

A Review on the Pharmacological Effects of Hormonal and Anti-diabetic Drugs in the treatment of Polycystic Ovary Syndrome

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

The Department of Pharmacy

Brac University
September, 2019

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Declaration

It is hereby declared that

1. The project submitted is my own original work while completing degree at Brac University.
2. The project does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The project does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Abstract

Polycystic ovary syndrome is one of the most common heterogenic disorder that affects 5-10% women during their reproductive age. The purpose of this project is to compare the effects of hormonal and anti-diabetic drugs associated with polycystic ovary syndrome. The effects of the anti-diabetic drugs such as metformin, pioglitazone, rosiglitazone, letrozole and hormonal drugs like ethinyl estradiol, estradiol valerate, cyproterone acetate, medroxyprogesterone acetate, deinogest, desogestrel, drospirenone on PCOS affected women have been emphasized in this investigation. In most of the cases, the combination of both hormonal and antidiabetic drugs have provided better results. This work has been focused on the laboratory assay techniques, results and discussion provided on these articles. The most advantageous effects of the available drugs have been emphasized in these work. The researchers and clinicians may be benefited from this project on the ground of selection of combination drugs for the treatment of PCOS.

Keywords: Endometrial Carcinoma; hyperinsulinemia; hyperandrogenism; drospirenone; metformin; anovulatory infertility.

Dedicated to my Parents

Acknowledgement

I would like to begin by thanking the Almighty, our creator, the source of our life and strength, knowledge and wisdom, for the blessings and mercy. All praises to Him and I am grateful to Him for blessing me with immense strength, patience and assistance whenever necessary to complete this project. This research would not have been completed without the assistance of the people who are gratefully recognized here.

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List of Acronyms

PCOS	Polycystic Ovary Syndrome.
IR	Insulin Resistance.
TT	Total Testosterone.
FAI	Free Androgen Index.
F-G score	Ferriman-Gallwey score.
LH	Lutenizing Hormone.
FSH	Follicle Stimulating Hormone.
SHBG	Sex Hormone Binding Globulins.

Chapter 1

Introduction

1.1 Polycystic Ovary Syndrome (PCOS)

There are lots of controversies associated with diagnostic features and defining criteria of PCOS. It has been defined as heterogeneous condition owing to the existence of numerous clinical symptoms. Some are found to be lean, whereas others are obese, many of them are resistant to insulin and many of them are not. The patients receive different kinds of treatment depending on the symptoms like obesity, infertility, hyperandrogenism and so on (Nawrocka & Starczewski, 2007). PCOS has been specified earlier by Stein and Leventhal in the year of 1935. It is the most common endocrine disorder, influencing around five to ten percent of ladies of childbearing age (Palomba et al., 2005). The presence of multiple cysts in the human ovary may be identified by ultrasound test relying on the possibility that the ovaries are 10 cm³ larger compared to healthy ovary and minimum twelve follicles are present which has been estimated as 2–9 mm in diameter. Alterations in the ovaries might be assured with either single or both of the human ovaries (Mitkov, Pehlivanov, & Terzieva, 2005).

Ovarian cysts classification:

A cyst may be a fluid-filled sac that grows within the ovary and typically disappears once after ovulation. There are differing kinds of cysts:

Follicular cyst -A follicular cyst grows in every month and contains a tiny egg inside it. The sac breaks throughout ovulation process and also the egg departs from the ovary. Typically the cyst may become larger than the traditional size that is up to three cm (slightly greater than one inch). If the sac that contains the egg does not rupture to release the egg, it will grow, anyplace in size from ½ inch (1cm) to four inches (10cm) across. Most follicular cysts may go away

within 2-8 weeks and do not induce pain. Giant cysts might or might not cause pain and/or pressure within the lower belly (on the facet wherever the cyst is) and typically takes longer to recover or removal. The health care supplier (HCP) might notice that the patient just carry a follicular cyst throughout a pelvic test or by a check known as ultrasonography.

Corpus luteum cyst – A corpus luteum cyst is ordinary and typically forms every month once after ovulation. This kind of cyst sometimes go away by itself during a few weeks, however it will typically take up to 3 months. A corpus luteum cyst will develop up to 3-4 inches across, and will bleed within the cyst or inside the patient's belly and may induce pain. (Health Guides, 2018, retrieved from <https://youngwomenshealth.org/2011/05/23/ovarian-cysts/>)

1.2 Epidemiological background of PCOS

Sufficiently the predominance of PCOS will rely upon the criteria developed to define this disease. Most probably, PCOS may arise earlier during puberty and progress through young age into adulthood (De Leo, Musacchio, Morgante, Piomboni, & Petraglia, 2006). Initially in White or Caucasians along with Black races, the prevalence of this disease has been studied. The investigation including two hundred and seventy seven ladies in Southeastern US, it has been primarily reported that the final prevalence of PCOS based on 1990 National Institute of Health criteria was 4.0% with no remarkable distinction between Whites and Blacks. Another investigation of one hundred and ninety two ladies on Lesbos Island in Greece, through providing medical tests without charging money, the predominance of PCOS has been found to be 6.8%. Subsequently, the predominance of clinically confirmed PCOS in random ladies of fertile age varies from six point five to eight percent employing the 1990 National Institutes of Health criteria. Since there are imprecisely sixty two million ladies aged 15–44 years within the US, this study has provided the estimation of at least 4–5 million ladies of fertility are affected by PCOS. However, various symptoms are related to exaggerated predominance of

PCOS, incorporating fatness, existence of insulin resistance, type one or type two diabetes mellitus, or oligoanovulatory physiological state. The predominance of PCOS additionally found to be greater between Mexican yankee compared to White/African yankee ladies. (Goodarzi & Azziz, 2006)

1.3 Pathogenesis of PCOS

- Hypothalamic-pituitary axis abnormalities cause impaired discharge of gonadotropin releasing hormone, luteinizing hormone has been induced by hypothalamus-pituitary axis irregularities leading to an increased androgenic hormone production by ovaries.
- The generation of surplus androgen has been facilitated by an enzymatic defect in the steroidogenesis of the ovaries.
- The reproductive and metabolic anomalies in polycystic ovary syndrome has been induced by resistance to insulin hormone (Escobar-Morreale, 2018)

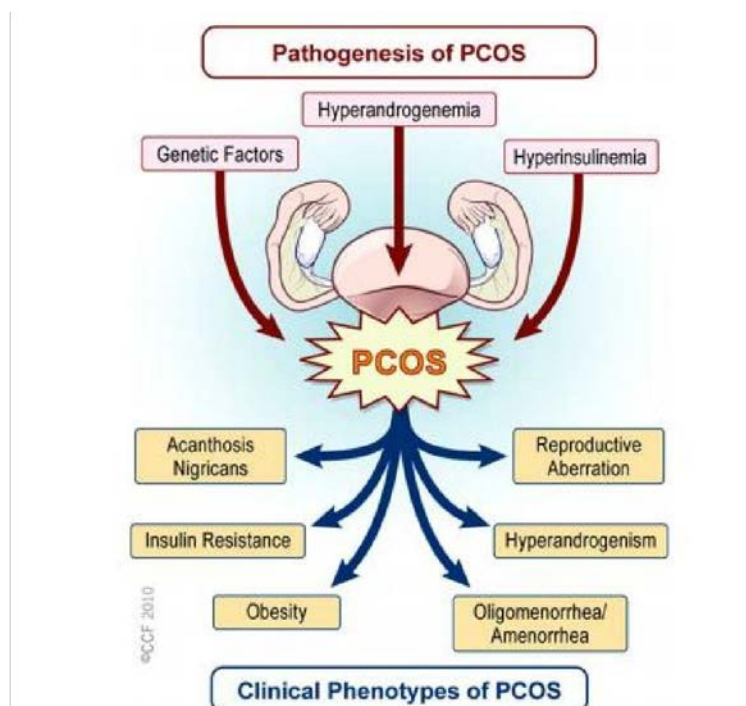


Figure 1: Schematic description of various pathological elements that induce PCOS in fertile women. (Yeon Lee, Baw, Gupta, Aziz, & Agarwal, 2010)

1.4 Symptoms of PCOS

In spite of several criteria being proposed for the determination of PCOS, oligoanovulation because of ovarian dysfunction keeps on being the critical element that makes this disorder the real reason for anovulatory infertility in civilized countries (Palomba et al., 2005). It is a heterogenic accumulation of indications with hereditary inclination, which consolidated in a diverse way, decide the assortment of clinical appearance. A portion of the patients have no bad things to say at all and the clinical signs are not discernible. Different patients appear expressive conceptive, hormonal and metabolic deviations.

In clinical practice, PCOS can be confirmed when at least two of the mentioned indications appeared:

- 1) Deficiency or absenteeism of ovulations,
- 2) Sonographic evidence of the presence of multiple cysts inside the ovaries,
- 3) Unbalanced menstruation (minimum rate of inadequate drainage: twenty nine percent, minimum rate of amenorrhea fifty one percent),
- 4) Along with, hyperandrogenism (mean occurrence of hirsutism is sixty nine percent)
- 5) Absenteeism of additional endocrinological status, for instance Cushing's syndrome, hypothyroidism, hyperprolactinemia, congenital virilizing adrenal hyperplasia (Mitkov et al., 2005)

Among those, obesity is considered to be the most complaining dominant symptoms of PCOS. Excess abdominal fat irrespective of body mass index has been found to be firmly associated with reduced sensitization towards the hormone insulin in PCOS women. The patients exhibit android type of adipose tissue ordination which is related to greater hazard of circulatory

system problems along with metabolic disturbances. The type of fatness is determined by raised waist perimeter > 88cm and waist-to-hip ratio > 0.8 (Nawrocka & Starczewski, 2007)

A table has been presented here including all the available definitions of PCOS (Goodarzi & Azziz, 2006). PCOS affected women recurrently suffer from the unpleasant impacts of metabolic issues, for instance, resistance to insulin hormone and impaired lipid profile.

A. Insulin resistance: Insulin resistance induces modifications in beta cell action to convey an elevated hazard of acquiring type II Diabetes Mellitus. Based on the premise of clinical or investigation criterion, insulin resistant PCOS patients may be separated from patients with ordinary sensitization to insulin hormone and those criteria combine the existence of fatness or acanthosis, insulin and glucose indices (Baillargeon, Jakubowicz, Iuorno, Jakubowicz, & Nestler, 2004).

B. Cardiovascular diseases: Cardiovascular risk indicators accumulate in PCOS affected women and their relatives, though the sign of an increased rate in cardiovascular morbidity or mortality in PCOS women is until now insufficient. Surplus androgen levels promote to the connection of this disorder with cardiovascular hazardous elements beyond resistance to insulin hormone and obesity (Nawrocka & Starczewski, 2007).

C. Hyperandrogenism: Neither obesity nor resistance to insulin hormone is responsible but hyperandrogenemia is the substantial factor of the prolonged carotid intima-media thickness (CIMT, a primary indicator of atherosclerosis) obtained in PCOS affected women (Luque-Ramírez, Mendieta-Azcona, Álvarez-Blasco, & Escobar-Morreale, 2009).

The definition of PCOS most frequently accepted nowadays are based on the researchers seminar patronized by the NIH in April 1990. (Table-1)

Table 1: Established criteria to define PCOS.

Definitions in different years	Defined criteria of PCOS
<p>NIH, 1990</p> <p>To incorporate all of the given symptoms:</p>	<p>Hyperandrogenism and/or hyperandrogenemia.</p> <p>Oligo-ovulation.</p>
<p>Homburg, 2002</p> <p>Patients represented with single or other of the given symptoms should complete ultrasound testing of the ovaries.</p>	<p>Hirsutism.</p> <p>Irregular menstrual cycles.</p> <p>Pimples</p> <p>Unproductiveness with chronic anovulation.</p> <p>If ultrasound test depicts presence of multiple cysts in ovaries, at that time the analysis of PCOS is assured. If this test fails then extra biochemical examinations have been carried out, and if any of the given indications is observed not to be normal then the further test of PCOS is endured.</p> <p>Raised testosterone.</p>
<p>ESHRE/ASRM (Rotterdam), 2003</p> <p>To incorporate two of the given symptoms as well as occlusion of related disorder:</p>	<p>Oligoamnenorrhea- or anovulatory infertility.</p> <p>Hyperandrogenemia.</p> <p>Multiple cysts in ovaries.</p>
<p>Modified NIH criteria to incorporate all of the given symptoms:</p>	<p>Excessive androgen levels.</p> <p>Impairment in the activities of the ovary.</p>

1.5 Pathophysiology of PCOS

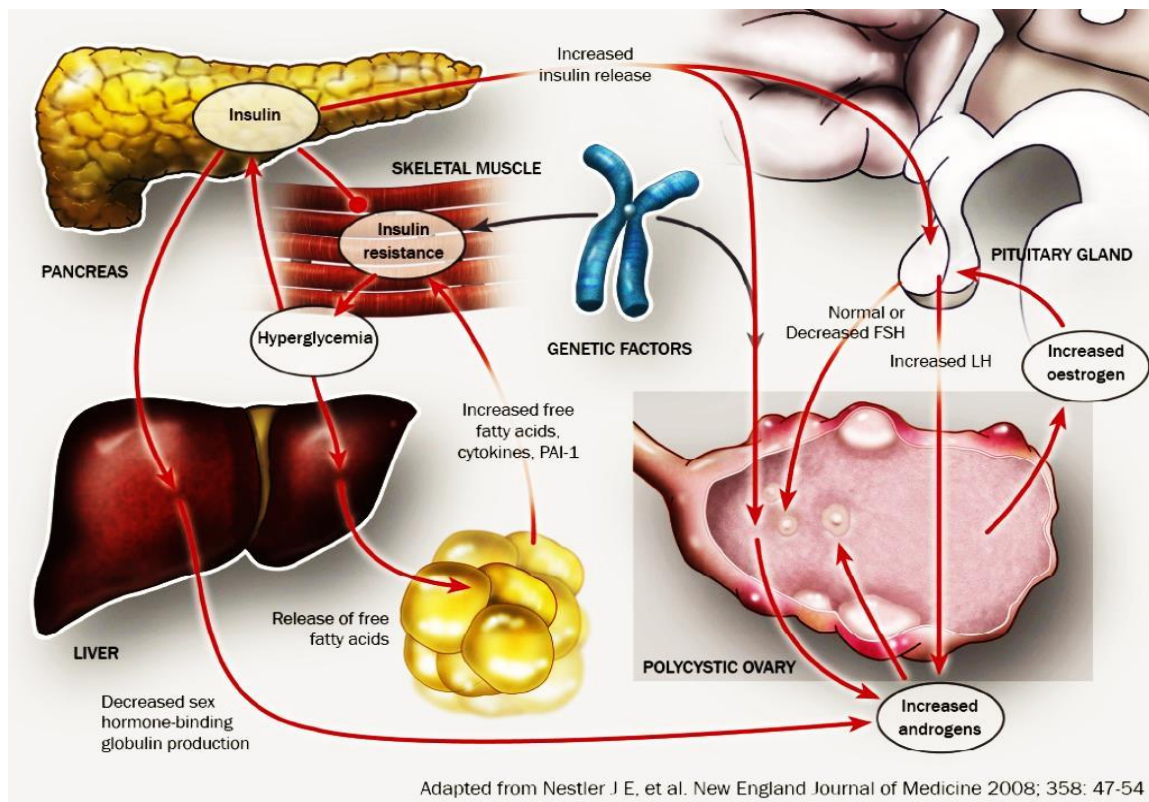


Figure 2: Pathophysiological attributes of PCOS.

1.6 Genetics and Heritability of PCOS

PCOS could be a frequent, complicated inherited illness. Collective ailments like asthma schizophrenia and type II diabetes, furthermore like PCOS, have shared a multiplex, versatile etiological background, a range of influencing genes, not only 1 gene, perform with environmental features to induce diseases. Family studies have represented the hereditary character of PCOS. Afterwards, several inhabitants' investigations have tried to determine genes that exert impacts on PCOS employing the participant's gene attitude. Family analysis have expressed that this disorder is considerably more predominant among relatives compared to normal people. Among first-degree female relatives (without hormonal treatment) of ninety three patients with PCOS, thirty five percent premenopausal mothers and forty percent of

sisters were conjointly affected with the ailment. These affected percentage considerably greater than the 6–8% discovered within the general population. Brothers of ladies with PCOS conjointly show atypical androgens: an examination of those brothers have shown prominent levels of DHEA-S. Among sisters of PCOS affected women, people having PCOS or excess androgen levels with regular menstrual discharge, showed lesser sensitization to insulin hormone compared to healthy sisters (evaluated through fasting insulin hormone as well as glucose estimations). Similarly, among Australian PCOS affected women and their families, excessive insulin levels has been observed to appear in sixty nine percent of all relatives, pointing out that this attribute was inherited. In investigations of PCOS affected women and their family members, concentrations of insulin release, estimated straightly through the recurrently used intravenous glucose tolerance test, expressed important hereditary connection, pointing out a genetic factor to impairment of b-cell functioning in PCOS(Goodarzi & Azziz, 2006).

1.7 Treatment options for PCOS

The suggested treatment options to treat each of these elements have been presented in Table - 2.

Table 2: Selected treatment options for PCOS.

Criteria	Available tests
Metabolic	<p>Check hazards for diabetes mellitus and cardiovascular disorders.</p> <p>Check for nonalcoholic fatty liver disease hazards.</p> <p>Communicate routine treatment modalities for instance nourishment and exercise.</p>

Table 2: Selected treatment options for PCOS.

Cycle Control	Determine menstrual discharges and hazards for hyperplasia of the endometrium. Administer treatment modalities for the prevention of hyperplasia of the endometrium: estro-progestin pills.
Psychosocial	Estimation of appetite and screening the body. Test if the patient is depressed. Convincing tension managing tricks. Ensure impartial support.
Cosmetic	Communicate the utilization of estrogenic pills to abolish hyperandrogenism, spironolactone may be suggested if it is not contraindicated. Communicate the utilization of enflornithine hydrochloride, treatment with laser light and electrolysis therapy.
Ovulation	Communicate pregnancy planning. Deliver treatments to raise the rate of ovulation: weight reduction, metformin. Suggest recommendation to refer into Reproductive Endocrinology Centre to be benefited with modern reproductive techniques.
Sleep Apnea	Examine to find out sleep apnea.

(Escobar-Morreale, 2018)

1.7.1: Lifestyle Modifications

Lifestyle modifications for instance a balanced diet and exercise have been recommended as first line treatment especially the PCOS affected women are obese. However, it has been found by several nonrandomized studies that reducing body weight through a balanced diet and regular exercise helps to enhance insulin resistance and ovulation frequency. In another study group, reduction of obesity of five to seven percent reduces the conversion of diminished

glucose tolerance into type II diabetes mellitus by fifty eight percent over a three year time period. the pharmacologic modalities of treatment of PCOS relies on the application of mainly 2 groups of medications, to be specific oral contraceptives incorporating a progestin of low androgenic property or alike antiandrogenic characteristics and insulin sensitizing drugs (Luque-Ramírez et al., 2007).

1.7.2: Oral contraceptives (OCP)

Medroxyprogesterone Acetate (MPA)

Alternate progesterone treatment shows the ability to restore menstrual regularity and can protect the endometrium in oral contraceptive contraindicated patients. Yet though it is well recognized that MPA show effects on the metabolism of glucose and lipid and in healthy postmenopausal women. The mechanism of action of MPA inappreciably differs from other OCPs. It prompts to remarkable suppression of LH and reduce testosterone synthesis. Additionally, secretion of removal of testosterone from circulation is raised as a result of liver enzyme induction activity (Özdemir, Görkemli, Gezginç, Özdemir, & Kiyici, 2008). Treatment with megestrol progesterone or MPA may enhance few cases of endometrial atypical hyperplasia (Li et al., 2014).

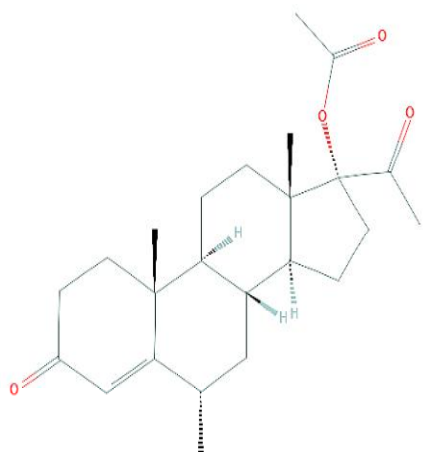


Figure 3: Medroxyprogesterone Acetate.

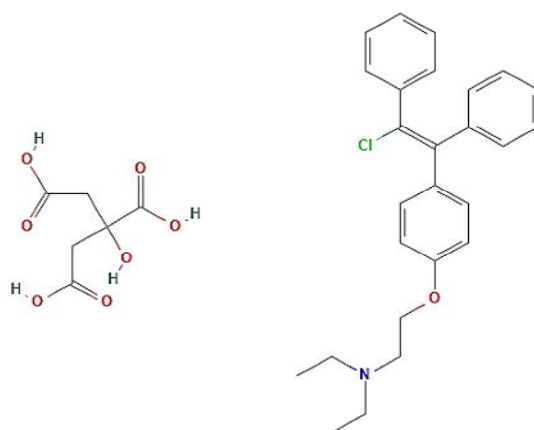


Figure 4: Clomiphene Citrate.

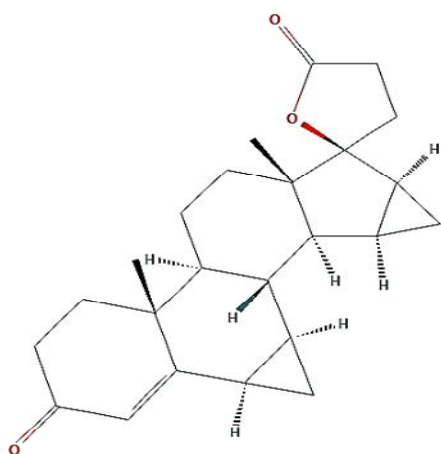


Figure 5: Drospirenone.

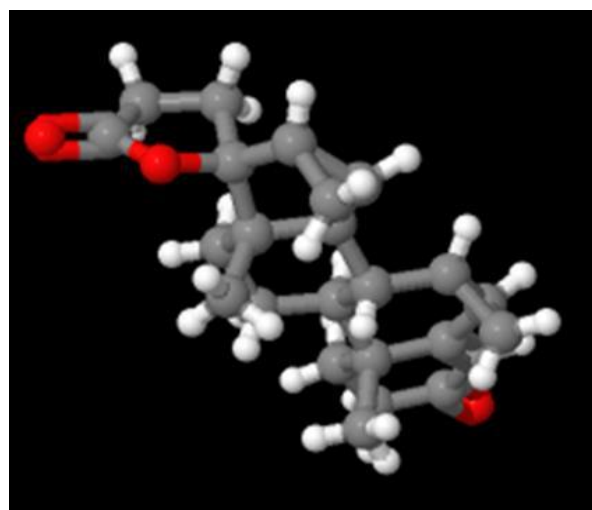


Figure 6: Drospirenone Crystal Structure.

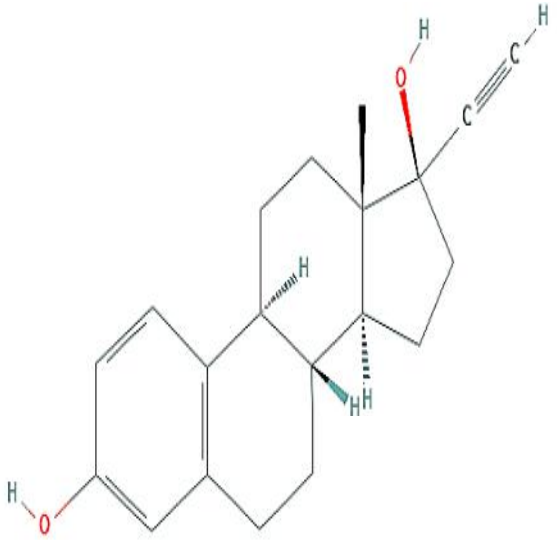


Figure 7: Ethinyl Estradiol.

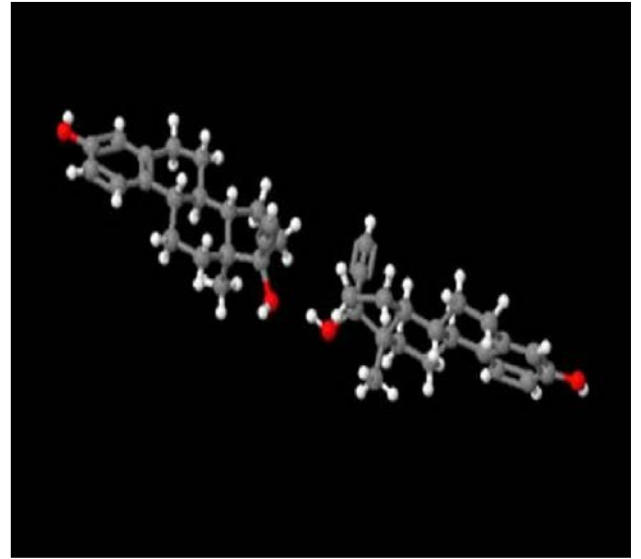


Figure 8: Ethinyl estradiol Crystal Structure.

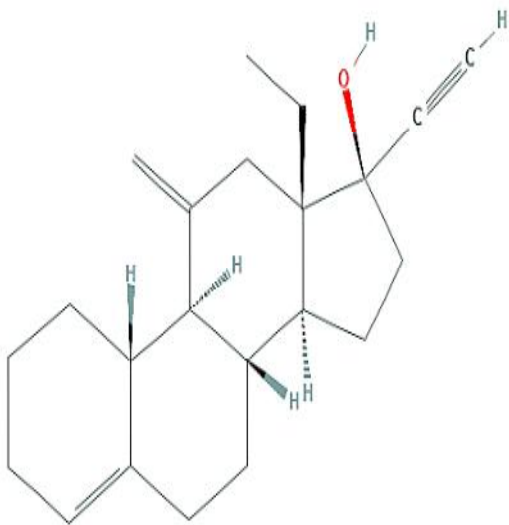


Figure 9: Desogestrel.

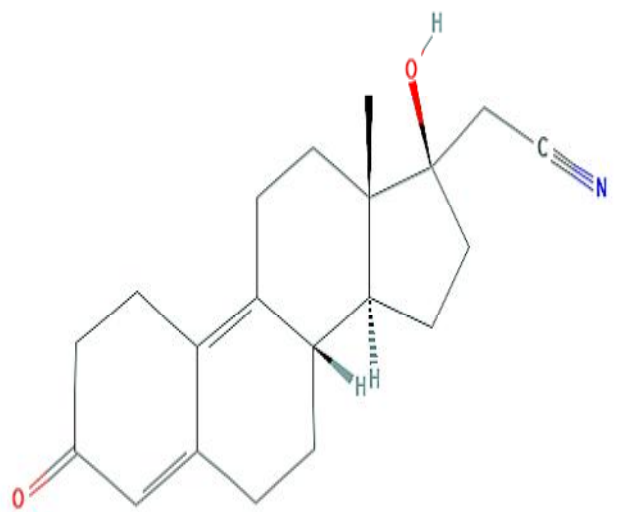


Figure 10: Dienogest.

Ethinyl Estradiol (EE) and Drospirenone (DRP)

The concentrations of sex hormone binding globulins have been raised by the hormone estrogen. As a result the plasma levels of circulating testosterone gradually diminishes. The progestin portion of the oral contraceptives delays 5 α -reductase action as well as antagonizes the androgen receptor. Another created combined oral contraceptives includes 30 mg ethinyl estradiol (EE) combined with another progestogen 3 mg drospirenone, DRP is created from 17 α -spiro lactone. Drospirenone antagonizes the activity of mineralocorticoid plus androgenic hormones, also has countless metabolic influences, together with capability to decline blood pressure as well as the weight of the body (Kriplani et al., 2010). Besides this, Drospirenone (DRP) included in ethinyl estradiol pill has potentiality to reduce hirsutism and testosterone levels (Fruzzetti et al., 2010).

Ethinyl Estradiol and Cyproterone Acetate (EE-CA)

This type of combination pill influences the regulation of High Density Lipoprotein-C metabolism. The enzyme plasma hepatic lipase is notably sex steroid sensitive estrogen reduces its functionality though androgen along with androgenic progestin raises it. The drug EE-CA shows its mechanism through the involvement of the above mentioned enzyme. (Luque-Ramírez et al., 2007). Although some commercial OCPs have been responsible to deteriorate the lipid profile, the improvement of hyperandrogenism in combination with the antiandrogenic capabilities of Cyproterone acetate and the Ethinyl Estradiol part of this pill may defeat this unwanted side effects, declaring its favorable effect in patients with PCOS. Besides this, EE-CA shows lesser impact on glucose tolerance and the drug is found to be well tolerated in general populations and no complain about its dropout rate.

Desogestrel (DSG)

Desogestrel (DSG) is considered as 3rd generation progestogen and is found to be both lipid-favoring and of lower androgenicity. Although OCPs including antiangiogenic hormones like cyproterone acetate or drospirenone are easily obtainable, those are too costly than DSG containing medicines. Hence, researchers have found it beneficial to utilize this pills in a resource-poor setting (Bhattacharya, Ghosh, & Basu, 2012) .

Estradiol Valerate (E2V) and dienogest (DNG)

Along with these, another oral contraceptive incorporating E2V (Estradiol Valerate) with highly powerful progesterone like dienogest (DNG). This is a new estrogen-progestin combination which exhibits less androgenic property, diverse administration timing for the hormones as well as different routes of drug delivery. DNG is produced from 19-nortestosterone that shows a higher progestogenic property inside the body, less binding affinity towards estrogen, glucocorticoid, mineralocorticoid and androgen receptors in outside the body along with anti-androgenic property both inside and outside the body. Besides this, DNG ensures decreased unwanted side-effects mainly towards lipoproteins, sensitization towards insulin hormone along with impacts on blood clotting factors (De Leo et al., 2013).

Clomiphene citrate

To induce ovulation in PCOS women, Clomiphene citrate is considered as first line medical care. The chemical structure of clomiphene and estrogenic products closely resembles each other. It acts by blocking the estrogen receptors(ER) and thus decreasing estrogenic impact. The combination of Ethinyl estradiol and Clomiphene citrate generates a beneficial endometrial response in barren ladies suffering with PCOS.

1.7.3: Insulin-Sensitizing Agents

The biguanide class of drug-Metformin:

The most familiar biguanide anti-hyperglycemic drug metformin has appeared to enhance hormonal plus metabolic abnormalities related to PCOS yet its actual process of function has been however in controversy. Metformin decreases plasma glucose level primarily through upgrading the utilization of carbohydrates in intestine, improving peripheral take-up of glucose along with sensitization towards insulin hormone preventing glucose synthesis by hepatocytes. However metformin alone takes too long time to show its effect. For this reason, it is combined with other hormonal drugs to achieve better and faster restoration of endocrine abnormalities (Mitkov et al., 2005).

Pioglitazone:

One of the thiazolidinediones group of drug, Pioglitazone has obtained its application in PCOS affected (women resistant to metformin or unsophisticated towards any kind of insulin sensitizers) (Ortega-González et al., 2005). through the attachment into the peroxisome proliferator-activated receptor (PPAR- γ) significantly in the adipose and muscle tissues, thiazolidinediones are implicated in the transcription of several elements concerned with the regulation of lipid and carbohydrate metabolism,. Troglitazone, rosiglitazone and pioglitazone are also found to be efficient in improving reproductive capability and come into sight to ensure in developing endocrine and metabolic irregularities (Lemay, Dodin, Turcot, Déchêne, & Forest, 2006).

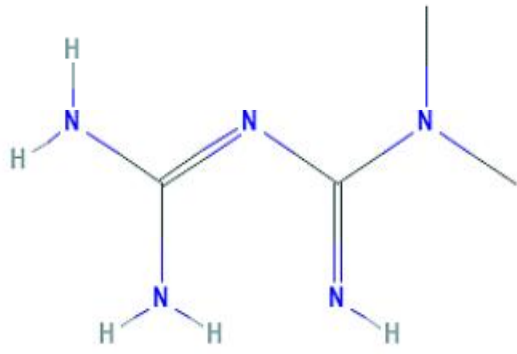


Figure 11: Metformin.

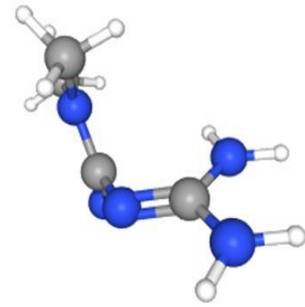


Figure 12: Metformin crystal structure.

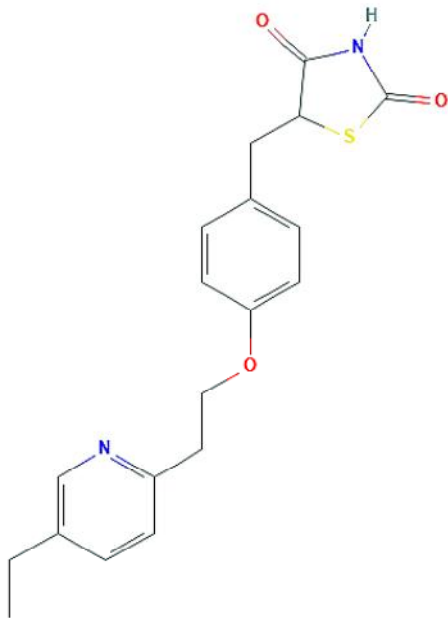


Figure 13: Pioglitazone.

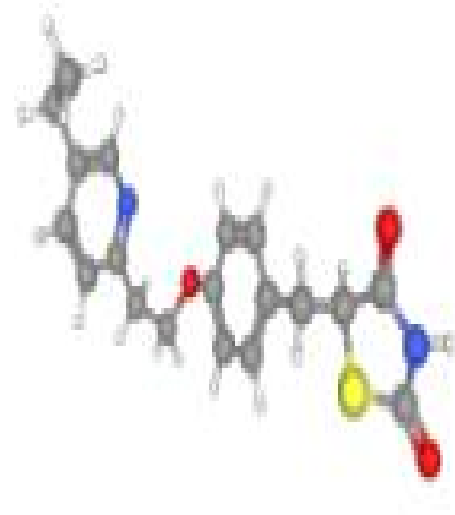


Figure 14: Pioglitazone crystal structure.

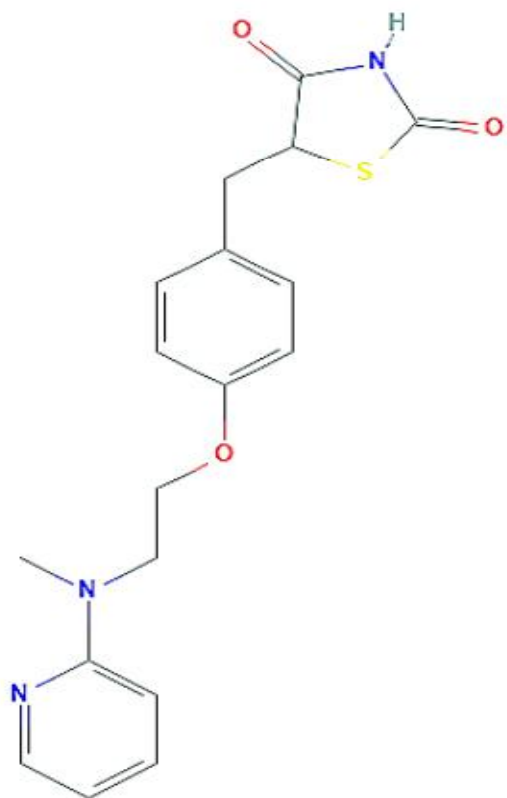


Figure 15: Rosiglitazone.

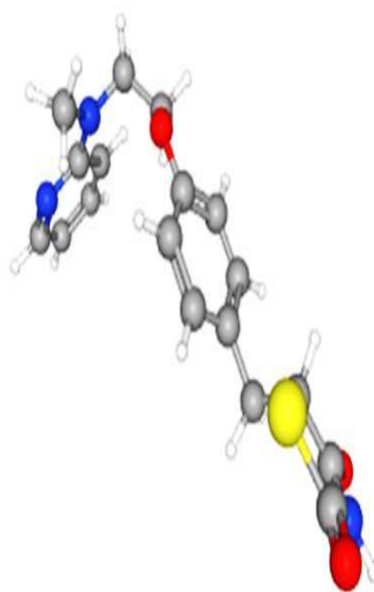


Figure 16: Rosiglitazone crystal structure.

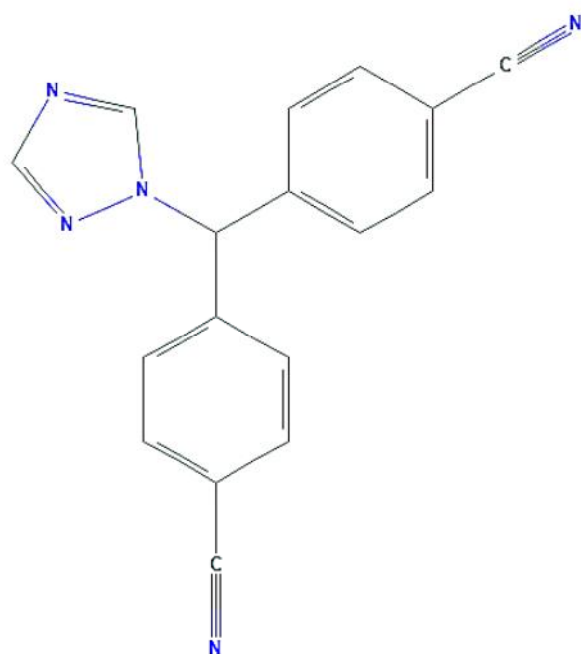


Figure 17: Letrozole.

Rosiglitazone:

At present, rosiglitazone which has a PPRA- γ agonistic action, is also a member of the TZD class, gained enormous importance in the treatment of insulin-independent diabetes mellitus. Differing from metformin, it is called as an actual insulin-sensitizing drug, triggers PPRA- γ (a receptor of hormone placed in the nucleus of the cell). Rosiglitazone performs by raising insulin sensitivity through a unique pathway with its impact on PPRA- γ . Through these pathways rosiglitazone affects the transcription of several genes involved in the homeostasis of lipid and glucose. Therefore the glucose uptake at the peripheral site and β -cell activity in the pancreas has been enhanced (Rautio, Tapanainen, Ruokonen, & Morin-Papunen, 2006).

Letrozole:

On the other hand, another drug named letrozole (an Aromatase inhibitor) has been found to be beneficial in clomiphene resistant patients. Instead of showing antagonistic effect, the drug letrozole exhibits agonistic activity of estrogen on endometrium. The mechanism of action of letrozole is that it prevents the transformation of androgen into estrogen to induce ovulation and thus it produce estrogen-absent conditions (Seyedoshohadaei, Tangestani, Zandvakili, & Rashadmanesh, 2016)

1.7.4: Anti-Androgen medical care

Spironolactone (50-100 mg double daily) has been proved to cure hirsutism efficiently. It is usually utilized in combination with oral contraceptives due to possessing supplemental effects to suppress the androgenic hormones (oral contraceptives) and is able to prevent androgen (spironolactone). However spironolactone is contraindicated in pregnant women as it shows potential teratogenic effects (Escobar-Morreale, 2018).

1.8: Endometrial Carcinoma risks for PCOS

Women with PCOS affected women are at greater risk of pregnancy loss and more severely for endometrial cancer. Indisputably the endometrial thickness is considered as one of the most significant part in treating infertility. If the endometrial thickness is lower than six to eight millimeter, the rate of pregnancy will be significantly low. Clomiphene citrate has been proved to decrease endometrial thickness and is an important area of interest to the researchers (Seyedoshohadaei et al., 2016). Malignant endometrial cells may be one of the most common deadly gynecological thread. Adolescent PCOS affected females are at greater danger of cancer of the endometrium. Besides this, PCOS ladies already suffering with hyperplasia of the endometrium, are four times mostly prone to be affected by the cancer of the endometrium compared to PCOS-unaffected women. It has been found by many researchers that modifications in endogenous hormone metabolism, insulin resistance (IR), and fatness may be related to endometrial cancer development. Meanwhile adolescent PCOS-affected females normally desire to continue their reproductive capacity, this tendency imposes a defiance to the research association to discover innovative plus conventional methods of treatment (like oral contraceptives) for the prevention and cure of the cancer of endometrium (Li et al., 2014).

Chapter 2

Results and Discussion

2.1 A study on the combined effect of metformin and EE-CA to treat endometrial carcinoma in PCOS ladies with insulin resistance.

Young aged women suffering with PCOS are at greater risk of acquiring endometrial carcinoma. That is why it has become utmost important to find out non-surgical medical treatment so that the reproductive capacity can be restored in young ladies. The human endometrium has been made up of glandular plus luminal epithelial cells, immune-defending cells, stroma with stromal fibroblastic cells, blood vessels and is responsive to steroid hormones. The balance between estrogen and progesterone coordinated function is required for the control of multiplications, differentiation, apoptosis along with secretion of stromal and epithelial cells. All these above cycles must function coordinately to assist the endometrium to act normally such as implantation, pregnancy, menstruation and repairment of endometrium. Nevertheless, the two steroid hormones occasionally perform in the opposite way that is estrogen induce hypertrophy and the hyperplasia and hypertrophy of endometrial cells has been induced by estrogen whereas progesterone prevent this estrogen-induced proliferation. The endometrium of PCOS ladies exhibits prolonged estrogen induction with lower or entirely absenteeism of progesterone stimulation. As a result, to prevent endometrial proliferation and restoration of menstrual regularity, progesterone contraceptive pills have been selected. Endometrial dysfunction that prompts to development of endometrial carcinoma has been associated with abnormalities in steroid hormone metabolism. The results of this article have claimed that regulation of the expression of estrogen receptor and progesterone receptor otherwise the induction of Estrogen Receptor- or Progesterone Receptor-mediated mechanisms are associated with the development of the cancer or hyperplasia of the endometrium of PCOS

affected women. The persistent exposure to estrogen might be accounted for the amplified endometrial expression of androgen receptor throughout the progression of hyperplasia of the endometrium of PCOS affected ladies. Besides this, IGF-1 signaling possibly responsible for the progression of cancer and hyperplasia of the endometrium of PCOS affected women. Five fertile PCOS women (age 26-32 years) are randomized to receive the combined drug including MET along with EE-CA. the duration of this study was six months. The results of this study ensures that adjoined treatment of EE-CA and MET can reverse the endometrial carcinoma into normal endometrial cells and reserve the fertility in PCOS insulin resistant ladies (Li et al., 2014).

Assay method:

1. Transvaginal ultrasound test has been performed to assess thickness of the endometrium.
2. Kidney and liver functionality have been also tested.
3. To ensure the presence of cervical cancer along with precancerous abrasions, a cytological test named as Cervical ThinPrep has been carried out.
4. Biopsy of the endometrium has been done by using hysteroscopy or endometrial curettage.

Result:

1. Endometrial carcinoma reverses into normal endometrial epithelial cells after combined treatment of six months.
2. Significant reduction in body weight, body mass index, plasma concentration of TT and FAI.
3. Remarkable increase in sex hormone binding globulins.
4. Important reduction in glucose and plasma insulin levels.

5. Notable reduction in HOMA-IR pointing out either a remarkable reduction in insulin resistance or an initiation of insulin sensitivity.

Discussion:

1. Ethinyl estradiol has been found to suppress ovarian estradiol synthesis by inhibiting the hypothalamic-pituitary-ovarian axis.

3. Through a dose-dependent modulation, metformin prevents cell propagation as well as initiates apoptosis of various cancer cells of the endometrium.

4. Metformin also reduces cell multiplications as well as induces apoptosis of the serous carcinoma of the uterus by preventing insulin/ IGF-I signaling pathway.

5. Obesity is accounted for developing endometrial cancer, however combined treatment method has found significant reduction of body weight and also in BMI.

2.2 A study on the impacts of OCP including ethinyl estradiol plus drospirenone versus desogestrel on PCOS-related abnormalities.

A prospective randomized test has been carried out to evaluate the potency of two oral contraceptives (drospirenone versus desogestrel combined with ethinyl estradiol) in PCOS ladies who non-desirous of pregnancy. Randomization: Sixty PCOS women (age 22.5 ± 4.7 years) have been divided into two groups. One is the study group [(EE) 30 mcg+ DRP 3 mg] and control group (EE 30mcg+ DSG 150 mcg] and the duration of this study has been six months. The data of this study has suggested that drospirenone incorporated OCP pill exhibits better results in terms of restoring menstrual cycles, anti-androgenic impact, BMI and blood pressure, good lipid values and beneficial hormonal plus glycemic profile in comparison with the desogestrel incorporated pill. In addition, to treat hirsutism in fat PCOS ladies, drospirenone- incorporated OCP is recommended as a good option (Kriplani et al., 2010)

Assay method:

1. microparticle assay technique has been used to estimate the level of total testosterone.
2. Enzyme immunoassay method has been used to analyze DHEA-S level.
3. Chemiluminescent immunometric technique has been used to determine SHBG levels.
4. ELISA immunoassay technique has been used to determine postprandial and fasting insulin levels.
5. Beckman Coulter CX9, CX4 auto-analyzer machine has been used to analyze all the lipid values.
6. Plasma glucose level has been determined through glucose oxidase technique using an auto-analyzer.

Result:

Table 3: Comparison between study and control group.

criteria	Study group	Control group
Menstrual cycle	regular 44.83% (13/30)	regular 17.24% (5/30)
hirsutism score	33.3% decreased	no change
BMI	Reduced by 0.52 kg/m ² (1.9%).	Increased 5.3%.
systolic and diastolic BP	1.6% Fall in systolic BP and 0.5% fall in diastolic BP.	Systolic BP 1.4% rises and diastolic BP 4.2% rises.
Ovarian volume:	Decreased by 7%.	Reduced by 5.3%.

Table 3: Comparison between study and control group.

Endometrial thickness:	13.8% reduced.	7.1% decreased.
Lipid profile- Total cholesterol:	Significantly increased by 8.5%.	Increased by 6.7%.
Triglyceride levels	Increased by 11.8%.	14.4% rises.
Mean LDL	Decreased significantly by 7.2%.	Significantly increased by 6.5%.
HDL levels	Significantly increased by 12.3%.	Significantly increased by 5.4%
VLDL levels	Increased by 14.2%.	Rises 16.9%.
SHBG levels	Elevated.	Elevated.
Free androgen index	Reduced.	Reduced.
FSH level	Decreased due to pituitary suppression.	Reduced due to suppression of pituitary.
LH levels	More reduction.	Less reduction.

Discussion:

1. Drospirenone has good control over menstrual cycles.
2. The median decline facial acne in drospirenone is 62% and 59% found in cyproterone acetate incorporated OCPs.
3. Drospirenone also reduced hirsutism score by 36.5%.
4. As the result of this study has found that body weight has been significantly decreased in drospirenone group, so it can be a good option to treat PCOS ladies who are reported as overweight in 41% of cases.

5. Besides this, the most important finding of this article is that drospirenone is able to significantly decrease endometrial thickness, ovarian follicle number as well as ovarian volume.
6. Finally, drospirenone exhibits enhancement in insulin sensitivity that correlates with other similar studies.

2.3 Impacts of estradiol valerate and dienogest on glucose metabolism in insulin resistant PCOS women.

Among other clinical manifestations, insulin resistance and obesity is the predominant factor in PCOS women. This study has utilized a newly developed OCP containing estradiol valerate (E2V) included with progestogen dienogest (DNG) where the amount of estrogen has been kept lower and quantity of progestogen higher. Twenty PCOS ladies (aged 18-33 years) have been randomized to receive E2V/DNG and is evaluated after three months. This outcomes of this study has claimed that natural form of estradiol along with nonandrogenic progestogen in the form of Qlaira preparations may be a good option in insulin resistant obese PCOS patients.

Result:

1. E2V/ DNG drug has been found to be well tolerated and complaints about side effects are less.
2. Glucose median values have been suggestively reduced in comparison to the pretreatment group.
4. Median index for insulin resistance (HOMA-IR) have remarkably reduced than median values before treatment. [HOMA before 3.92 and after 1.30]

Discussion:

1. The most interesting beneficial outcome of this study is that median body mass index have not changed after this treatment so it ensures those ladies who often worry about gaining weight from using OCPs.
2. Besides affecting carbohydrate metabolism, the combined E2V/DNG also develops insulin sensitivity which is contributed to the natural form of estradiol (E2V) and to the antiandrogenicity of deinogest.
3. In contrast, though oral administration of E2V induces hepatic enzymes and globulins in primary first pass, the impact is lower than the biologically equal dose of ethinyl estradiol.
4. Progestins with antiandrogenicity or less androgenic status cause an increased insulin half-life whereas the androgenic progestin exactly shows the opposite effect.
5. Successively, DNG has a potential impact on endometrium of PCOS women and can modify the estradiol causing insulin resistance.

2.4 Impacts of ethinyl estradiol along with drospirenone plus medroxyprogesterone acetate and in PCOS women.

63 PCOS ladies have been divided into two groups (MPA and EE/DROS) to receive those drugs and is tested after six months to assess the clinical, metabolic and hormonal effects of these drugs in PCOS patients. The results of this study have suggested that both drugs have different effects in terms of clinical, biochemical parameters in PCOS women. MPA can provide better control of menstrual cycle in patients without dyslipidemia or hyperinsulinemia and bring about significant changes in serum hormone levels related to hyperandrogenism. Additionally, MPA might enhance skin manifestations associated with hyperandrogenism and is good option in OCP contraindicated patients (Özdemir et al., 2008).

Assay method:

1. Synchron LX20 machines are employed to assess lipid and glucose levels.
2. BioRad Hemoglobin Analyzer has been used to determine HbA1c by High-pressure liquid chromatography analysis.
3. Chemiluminescent immunoassay with original DPC reagents has been used to measure the levels of FSH, LH, E2, prolactin, DHEAS, TT, and SHBG.
4. Radioimmunoassay kit has been utilized to determine the levels of free testosterone.

Result:

Table 4: Comparison between MPA and EE/DROS.

criteria	MPA	EE/DROS
Lipid & carbohydrate metabolism	No significant alterations.	Significant rise in HDL-C levels, reduction in triglycerides, VLDL-cholesterol levels. Total cholesterol along with LDL cholesterol have not been changed.
Blood LH and testosterone levels	Fall.	Rise. ($p < 0.001$ for all parameters).
Free androgen index, seborrhea, acne	Decreased FAI ($P = 0.02$), rise in acne and seborrhea score. Improvement in skin manifestations.	Decreased acne, seborrhea, hirsutism, hair loss, FG score.

Table 4: Comparison between MPA and EE/DROS.

Menstrual cycle control	Good.	
OCP contraindicated patients	Good option.	
To treat hyperandrogenism	Beneficial.	

Discussion:

1. The second generation progestin, MPA has been proved to restore menstrual cycle of women with PCOS. When combined with estrogens, MPA tends to reduce insulin sensitivity remarkably in in-vivo study.
2. By the study of Bagis et al. short time treatment with PCOS in a small sample size exhibits a favorable impact on the values of HOMA and also enhanced insulin sensitivity of PCOS women.
3. In this present study, mean fasting insulin levels have been raised in EE/DROS group.
4. In the EE/DROS group, the triglycerides, HDL and VLDL cholesterol levels have been greatly raised.
5. Notable reduction in LH, TT and FAI has been reported in MPA group.
6. Remarkable reduction in TT and sex hormone binding globulins have been reported in EE/DROS group.
7. MPA has become successful to reduce acne and seborrhea scores. However, long term treatment with MPA might be efficient to permanently remove hirsutism in PCOS ladies.

2.5 Impacts of EE-CA on metabolic indices, distribution of fat as well as carotid intima media thickness in PCOS patients.

Carotid intima media thickness (CIMT) is a marker of greater cardiovascular hazards, has been found to be raised in PCOS affected women in comparison with the control group. This CIMT is a profound noninvasive indicator of preliminary atherosclerosis as well as distinct forecaster of cardiovascular disorders. This drug may lead to the development of arterial plaque which is raised in PCOS women yet earlier in puberty. Besides this visceral fat which a supplier is of cytokines and various hormones initiating a pro-inflammatory condition like the induction and development of atherosclerosis. This analysis has aimed to find out the impacts of EE-CA on metabolic hazards and distribution of fat in body of PCOS women. Thirty PCOS women (age: 24.4 ± 6.3 years) have been administered EE-CA and is evaluated after 6-months. The results of this investigation have suggested that this drug combination is an effective method of treatment in hirsutism and regulation of menstrual cycle. Though this drug have become able to reduce subcutaneous fat thickness after six months of follow-up, it has failed to exert any favorable impact on thickness of visceral fat along with metabolic and cardiovascular hazard parameters (Karabulut, Demirlenk, & Şevket, 2012).

Result:

- a) FG score as a marker of clinical hirsutism have been reduced remarkably ((mean \pm SD; 12.0 ± 3.4 vs 9.1 ± 3.3) and serum testosterone levels have become notably lower (0.59 ± 0.24) after six months of EE-CA treatment.
- b) No significant impact on carotid intima media thickness, insulin resistance and lipid values have been found.

- c) Though the blood concentrations of LH and DHEAS have also reduced with treatment of EE-CA, this result is not statistically important.
- d) Only decrease in subcutaneous fat thickness have become statistically important.

Discussion:

1. The results of this study have claimed that EE-CA is an effective drug to treat hyperandrogenism and irregular menstrual cycles.
2. Moreover, the analysts had not found any degradation in carbohydrate metabolism after six months according to the studies of Falsetti et al. and Luque-Ramirez et al. Hence, metformin should be prescribed along with EE-CA in insulin resistant PCOS patients.
3. No significant impact on lipid profile and cholesterol: HDL ratio have been reported.
4. No statistically important changes in carotid intima media thickness have been reported after six months of treatment.
5. In contrast to the common thoughts blaming oral contraceptives about weight increase, this study have not found any important changes in weight in the participants.

2.6 Impacts of EE/CA on endothelial function in adolescence lean PCOS affected ladies.

This is a pilot study to evaluate the impacts of EE/CA on endothelial function assed by indices of hyperandrogenism, brachial artery flow-mediated dilation (FMD), and insulin resistance of PCOS women. Long before structural vascular lesions appeared, endothelial dysfunction have been represented earlier in the progression of atherosclerosis. Reduced FMD has been associated to various risk elements and is an important predictor of raised cardiovascular diseases. Reduced FMD has been exhibited by non-obese PCOS ladies earlier and is related to

insulin resistance or hyperandrogenism. Hyperandrogenism not insulin resistance is responsible for endothelial dysfunction in lean PCOS ladies. Androgen reduction might be associated with enhanced endothelial function in PCOS affected patients through improvement of insulin resistance that is related to impaired endothelial function. Thirteen adolescent lean PCOS ladies (30 years old, Body Mass Index 530 kg/m²) have been administered EE-CA and is assessed for six months. Fourteen age- and body mass index (BMI)-equalized PCOS-unaffected women participated as control group. The results of this study ensures that treatment with ethinyl estradiol and cyproterone acetate reverts endothelial dysfunction in adolescent, lean PCOS ladies through enhancing hyperandrogenism (Naka et al., 2011).

Assay method:

1. The hexokinase method using a glucose analyzer has been employed to assess blood glucose levels.
2. Microparticle enzyme immune-analyzer has been used to determine insulin levels.
4. Enzymatic colorimetric technique has been used to measure total serum HDL-C and triglycerides values.
5. Microparticle enzyme immunoassays have been used to measure LH, estradiol and FSH levels.
6. Chemiluminescent microparticle immunoassay has been used to assess total testosterone levels.

Result:

1. Hyperandrogenism indices (total testosterone Ferriman-Gallwey score, free androgen index) LH: FSH values, LH have raised significantly in PCOS ladies.
2. Whilst concentration of SHBG have raised in control group.

3. FMD and NMD have reduced remarkably in PCOS affected group in comparison to control group.
4. After reconciliation for the dissimilarities found among groups in insulin resistance indices along with FMD have been yet remarkably less in PCOS affected group.

Discussion:

1. EE-CA pill have been well tolerated and no adverse effects have been observed.
2. It has been noted earlier that reduced NMD found in the investigated PCOS affected ladies has been one more vascular abnormal problem recommending degraded endothelium-independent vasodilation.
3. Although insulin resistance has been suspected to be the major forecaster of decreased FMD in overweight PCOS affected women whereas hyperandrogenemia (determined through raised free androgen index) might be significant in non-obese PCOS affected ladies.
5. The combination of ethinyl estradiol with cyproterone acetate is found to reduce the levels of a biochemical index of endothelial dysfunction known as asymmetric dimethylarginine (ADMA), in both overweight and lean PCOS ladies, whereas EE/CA has appeared as impartial on FMD in overweight PCOS ladies.
6. Treatment with EE/CA remarkably raises the levels of total cholesterol predominantly by raising HDL-cholesterol levels, which predicts to exert a protective impact on the cardiovascular system, have stayed perfectly in ordinary limited values. These impacts might be described through the estrogen activity of the ethinyl estradiol part mediated by a first pass impact in the liver.

2.7 Impacts of metformin versus ethinyl-estradiol in addition to cyproterone acetate on ambulatory blood pressure observing and carotid intima media thickness in women with the polycystic ovary syndrome.

In PCOS affected women, risks of cardiovascular disorder are associated with impaired insulin resistance, obesity, abnormal lipid profile, androgenic excess and so on. The target of this clinical trial is to monitor the effects of an antiandrogenic low dose oral contraceptive named ethinyl estradiol plus cyproterone acetate versus an insulin sensitizer metformin on ambulatory blood pressure (ABPM) and carotid intima media thickness (CIMT) in PCOS affected women. To the authors' best information, their study is the primary investigation to think about the effect of the two primary medication treatments accessible for PCOS, to be specific, oral contraceptives and insulin sensitizers, on the 24-hour ambulatory blood pressure profiles and CIMT of these patients. 34 women, age between 23-25yrs and BMI: 28-34KG/M² have been randomized to oral treatment with metformin or with ethinyl-estradiol plus cyproterone acetate drug for 24 weeks. The results of this study has suggested that metformin reduces daytime blood pressures whereas EE-CA have showed the inverse impact. PCOS affected patients who presents a family history of hypertension or who are at greater risk of cardiovascular diseases, the safer blood profile of metformin should be taken into consideration while prescribing oral contraceptives. Besides this, normalization of CIMT estimations has been achieved from both of these drugs (Luque-Ramírez et al., 2009).

Result:

Ambulatory Blood Pressure Monitoring Parameters

Following 24 weeks of treatment, and contrasted and standard estimations, EE/CPA increased though metformin diminished the normal systolic, diastolic, and mean blood pressure daytime measurement. These contrary impacts of the medications on blood pressure have not been

found during the night time. The valuable impact of metformin on blood pressure parameters was particularly apparent in the two hypertensive patients who finished the investigation also, had normal blood pressure values following 24 weeks of treatment with this medication.

Table 5: Comparison between EE/CPA and MET treated groups.

Nocturnal decrease in blood pressure	EE/CPA n= 15	Metformin (intention-to treat analysis, n =19)	Metformin (patients completing the study, n =12)	Metformin (lost to follow-up, n =7)
Systolic blood pressure (%)	9.4 ± 8.4	7.3 ± 9.3	10 ± 10	3 ± 7
Diastolic blood pressure (%)	14.0 ± 7.6	12.6± 9.4	16± 10	8 ±5
Mean blood pressure (%)	12.0 ± 7.4	10.0 ± 9.1	13 ±10	5± 4
Carotid intima media thickness (mm)	0.41± 0.01	0.40 ± 0.01	0.37± 0.08	0.46 ± 0.01b

Carotid Intima-Media Thickness Estimations:

At benchmark, four (27%) of the patients designated to EE/CPA and three (16%) of the patients designated to metformin had rising CIMT values (see Table 2). Considered as an entire, both the patients dispensed to EE/CPA (0.41 ± 0.01 mm) and to metformin (0.40 ± 0.01 mm) had expanded CIMT values contrasted with the nonhyperandrogenic control women. Standardization of the expanded CIMT at pattern was watched just in one of the patients treated with EE/CPA, while two of the patients giving typical CIMT at pattern created raised CIMT estimations after treatment with metformin.

Table 6: Blood pressure values of EE/CPA and MET treated groups.

Criteria	EE/CPA n= 15		Metformin (patients completing the study, n =12)		Metformin (intention-to-treat analysis, n =19)	
	Baseline	24 weeks	Baseline	24 weeks	Baseline	24 weeks
Hypertension	2 (13)	5 (33)	2 (18)	0 (0)	5 (28)	3 (17)
Isolated daytime hypertension	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Isolated nighttime hypertension	2 (13)	2 (13)	1 (9)	1 (9)	3 (17)	3 (17)
Nondipper pattern	5 (33)	6 (40)	3 (27)	4 (36)	10 (56)	11 (61)
Increased CIMT values	3 (20)	2 (13)	0 (0)	2 (17)	3 (16)	5 (26)

The outcomes of this study unmistakably demonstrate that the antiandrogenic oral contraceptive EE/CPA prompts a gentle increment in the normal daytime systolic, diastolic, and mean blood pressure estimations of PCOS patients, while metformin diminishes them. The expansion in daytime blood pressure came about too in an expansion in the normal 24-hour systolic and diastolic blood pressure, be that as it may, in actuality, such differential impacts of these medications on blood pressure have not been observed during the night time.

Discussion:

- a) Despite the fact that the issue of hypertension in PCOS is as yet disputable, they have recently demonstrated that variations from the norm in the 24-hour ABPM recordings are available in the same number of as thirty percent of PCOS affected women and that irregularities generally reliant on the simultaneousness of fatness in these PCOS patients.
- b) Too, PCOS affected women showing raised CIMT values in comparison to nonhyperandrogenic ladies, demonstrating the nearness of subclinical atherosclerosis, yet

this specific finding is related with androgen abundance and not with excess body weight or insulin resistance.

- c) The conceivable favorable impacts of metformin on blood pressure have been recently announced not just in PCOS women yet in addition in patients with type 2 diabetes, a finding that may be connected, at any rate halfway, to the reduction in vasoconstrictor particles, for example, endothelin-1 in PCOS women who have received metformin.
- d) The stamped reduction in serum androgen levels accordingly to EE-CA would have been required to bring about a decline in blood pressure parameters.
- e) The antiandrogenic impact of EE-CA , and the improvement of both hyperandrogenemia what's more, insulin resistance with metformin, may clarify the enhancement of CIMT in the participants on the grounds that androgen overabundance is the principle determinant of the raised CIMT in PCOS affected women autonomous from being overweight what's more, insulin resistance.
- f) Likewise, administration of metformin in PCOS patients brings about a decline in the serum levels of advanced glycated end products (AGEs), on the grounds that these particles encourage the multiplication and movement of smooth muscle cells toward the vascular intima, the lessening in the serum concentration of advanced glycated end products moreover might have been identified with the lessening in CIMT found in this study.

2.8 A study on the impacts of metformin, rosiglitazone and estradiol-cyproterone acetate on non-obese women with polycystic ovary syndrome.

Thiazolidinedione like rosiglitazone have achieved extensive utilization to treat type II diabetes mellitus and other insulin resistant conditions prevalent in PCOS patients. Besides this, oral contraceptives like estradiol-cyproterone acetate (ECA) combinations have been also proved

as efficient in protecting the human endometrium, restoring menstrual cycles as well as enhancing the hirsutism or acne conditions through reducing ovarian androgen synthesis. The purpose of this study is to analyze the biochemical, clinical and hormonal alterations in PCOS women after administering MET, ECA and ROSI drug. 94 PCOS women have been divided into MET- group (n =47), ROSI-group (n= 14), and (ECA) (n =33) and is evaluated after 4 months. The results of this study have claimed that in enhancing menstrual cycles and decreasing serum free-testosterone levels estradiol-cyproterone acetate is more efficient compared to insulin sensitizers like metformin or rosiglitazone. Besides this, in decreasing fasting insulin levels and reducing triglyceride levels, an insulin-sensitizer like metformin is more potent compared to estradiol-cyproterone acetate OCPs (Cetinkalp et al., 2009).

Assay method:

1. Immunephelometric technique has been used to assess serum levels of hs-CRP.
2. An automated analyzer has been used to determine serum levels of total cholesterol, LDL-C and HDL-C, triglyceride, aspartate aminotransferase (AST), alanine aminotransferase, and – glutamyltransferase.
3. Chemiluminescent immunometric assay has been used to determine blood levels of insulin.

Result:

Table 7: comparison between MET, ROSI and ECA treated groups.

criteria	MET	ROSI	ECA
Weight loss	More lost. Weight= 1.26) than patients in other two groups. BMI greatly decreased.	Not significantly changed. Weight = 0.23 for ROSI group.	Not significantly changed. Weight = 0.03 for ECA group).
insulin	Reduced at dose up to 1500 mg/d.	No change.	No changed.
Drug recommendation based on disease state	Hyperandrogenism	Hyperinsulinemia	
TCL, LDL-C	TCL level significantly decreased. HDL level not changed. Total cholesterol and LDL-C has been significantly reduced.	High HDL level.	High LDL and cholesterol level. HDL level not changed.
Hs-CRP levels	Decreased hs-CRP levels.	FALL in serum CRP.	
Fibrinogen concentrations	No change in fibrinogen levels.	Rise in fibrinogen levels.	No Change in fibrinogen levels.
DHEA-S levels	Significantly increased.	Significantly increased.	
Menstrual patterns	Improved.		Improved, fall in serum TT levels.

Discussion:

- a) Metformin is effective in enhancing metabolic irregularities, enhances menstrual cycles and in reducing androgens.
- b) On the other hand, ECA is efficient in reducing androgen levels and enhancing hirsutism score.
- c) Hs-CRP is a potent autonomous cardiovascular risk indicator in PCOS ladies. Metformin is effective in decreasing hs-CRP level and other two drugs have also showed effects on inflammatory indicators like hs-CRP and fibrinogen. Irregularities in blood coagulation and fibrinolytic pathway may also lead to progression of cardiovascular diseases.
- d) Estradiol shows a positive impact on fibrinogen concentration by an increase in fibrinogen levels in postmenopausal ladies and a decrease in fibrinogen levels during OCP therapy as well as hormone replacement therapy.
- e) In another investigation, rosiglitazone tends to reduce alanine aminotransferase activity and serum CRP concentrations.
- f) The adrenal cortex releases endogenous steroid hormones like dehydroepiandrosterone (DHEA) and its sulfated compound DHEA-S which dominate glucose tolerance and insulin sensitivity in humans. However it has been observed that the glucose tolerance along with blood levels of DHEA-S concurrently reduces with aging. Decreased DHEA-S concentrations have been found to be related to insulin resistance anomalies.
- g) It has been showed by Kazerooni et al. that metformin resulted in an enhancement of hyperandrogenism and its clinical symptoms in overweight PCOS ladies whose DHEA-S concentration is increased.

2.9 A study to evaluate the impacts of a thiazolidinedione (rosiglitazone) versus an estrogen-progestin oral contraceptive (ethinyl estradiol/cyproterone acetate) having a direct anti-androgenic property followed by their subsequent combinations on the metabolic and endocrine abnormalities in PCOS women.

Among the other available anomalies of PCOS, insulin resistance is more frequent and is associated with many of the symptoms complained by PCOS affected patients. The percentage of IR in PCOS women have been found to be $\geq 50\%$ among overweight and lean women. In conditions of IR, insulin sensitizing agents like rosiglitazone have been used to decrease insulin levels. Excess insulin level is responsible for the anomalies like anovulation, hyperandrogenaemia, obesity and dyslipidemia. On the other hand to prevent excess ovarian androgen production and hirsutism score and to regulate endometrial growth and uterine bleeding, oral contraceptives like ethinyl estradiol/cyproterone acetate has been used for long times in patients where pregnancy is not desired. This study is designed to assess the effectiveness and probable interaction between thiazolidinedione and estrogen-progestin pill on endocrine and metabolic problems of PCOS women. 28 participants (age 18-45 years) in whom increased insulin concentration have not normalized after four months of diet control have been divided into two groups: Group A(ROSI) and Group B(EE/CPA) and have been randomized to receive rosiglitazone and ethinyl estradiol/cyproterone acetate for six months. After that the group A have received EE/CPA and group B have received ROSI for another six months.

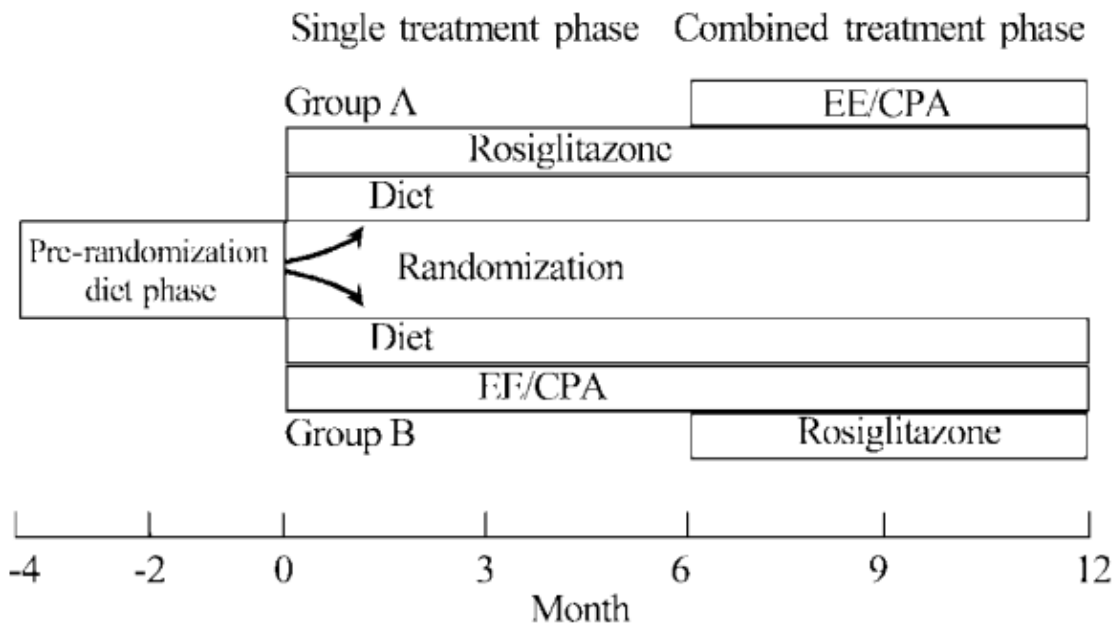


Figure 18: Illustration of consecutive treatment periods during the study method.

The results of this analysis have suggested that when utilized as individual drug, rosiglitazone principally decreased insulin resistance while the OCP have normalized insulin levels and enhanced hirsutism score. Interestingly, the combined treatment have provided integral benefits beyond antagonizing impacts, remarkable side-effects or alterations in safety manual (Lemay et al., 2006).

Assay method:

1. Electrochemiluminescence immunoassay has been used to determine thyroid-stimulating hormone, prolactin, LH, FSH, estradiol and progesterone.
2. Solid-phase ¹²⁵I radioimmunoassay has been used to determine the levels of total testosterone, androstenedione and dehydroepiandrosterone sulphate (DHEA-S).
3. Double antibody ¹²⁵I radioimmunoassay has been used to determine the levels of 17 α -Hydroxyprogesterone.

4. Immune-radiometric technique has been used to determine SHBG levels utilizing monoclonal antibodies.

5. Homologous radioimmunoassay method has been used to assess insulin levels using an antibody with low cross-reactivity to pro-insulin.

6. Enzymatic colorimetric reactions have been used to measure the levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) using a chemistry analyzer.

Result:

Table 8: Comparison between ROSI and EE/CPA treated groups.

criteria	Group A(ROSI)	Group B(EE/CPA)
1. Insulin, HOMA, QUICKI	Reduced.	No change in insulin levels. Combined treatment reduced insulin levels.
2. lipid profile	No change.	Raised HDL-C and Apo A.
3. Androstenedione, FAI and SHBG	Reduction of androstenedione and FAI and slight rise of SHBG.	Remarkable rise in SHBG and suppression of FAI levels. Decrease in total testosterone and DHEA-S.

Table 9: Total estimation of efficacy of evaluated criteria (mean± SEM) of combination of group A and Group B.

Criteria	Baseline Mouth 0	End Of Treatments Mouth 12	P
Glucose (mmol/l)	5.1 ± 0.1	4.8 ± 0.1	0.014
Insulin (pmol/l)	166 ± 14	101 ± 11	< 0.001
HOMA	5.93 ± 0.62	3.61 ± 0.41	< 0.001
QUICKI	0.299 ± 0.004	0.324 ± 0.007	0.001

Table 9: Total estimation of efficacy of evaluated criteria (mean± SEM) of combination of group A and Group B.

AUC glucose (mmol/l.h)	5.3 ± 0.8	5.1 ± 0.9	0.863
AUC insulin (pmol/l.h)	1942 ± 268	1205 ± 126	0.009
Triglycerides (mmol/l)	1.28 ± 0.14	1.76 ± 0.21	0.006
Total cholesterol (mmol/l)	4.41 ± 0.22	4.88 ± 0.22	0.050
LDL-cholesterol (mmol/l)	2.63 ± 0.19	2.48 ± 0.19	0.373
HDL-cholesterol (mmol/l)	1.19 ± 0.05	1.59 ± 0.09	< 0.001
Apolipoprotein A (g/l)	1.28 ± 0.04	1.74 ± 0.07	< 0.001
Apolipoprotein B (g/l)	0.88 ± 0.06	0.94 ± 0.05	0.111
Testosterone (mmol/l)	2.14 ± 0.18	1.49 ± 0.19	0.009
Androstenedione (mmol/l)	11.8 ± 0.6	8.6 ± 0.9	< 0.001
DHEA-S (pmol/l)	6.99 ± 0.79	4.40 ± 0.56	0.001
SHBG (mmol/l)	16 ± 1	143 ± 18	< 0.001
Free androgen index	14.3 ± 1.3	1.6 ± 0.4	< 0.001
Hirsutism score	17.2 ± 1.1	12.2 ± 0.4	< 0.001

The data have been analyzed by paired t-test.

HOMA= homeostasis model assessment; QUICKI=quantitative sensitivity check index.

AUC=area under the curve; LDL=low-density lipoprotein; HDL=high-density lipoprotein.

DHEA-S=dehydroepiandrosterone sulphate; SHBG=sex hormone-binding globulin.

Discussion:

1. The changes evoked by every drug are equal whatever one drug have been administered before or after the other in their subsequent combination.

2. Though there have been no alterations in anthropometric estimations, extremely important enhancements on metabolic (except for raised TG) and androgen indices have been discovered after finishing the treatment while outcomes of group A and B have been combined.
3. Significantly, the complementary helpful impacts of each drug have been also without remarkable side-effects or alterations in safety measures.
4. Overall, data in overweight PCOS ladies point out that the normal dose of every thiazolidinedione administered singly is efficient in reducing insulin hormone however has numerous efficacies on testosterone hormone and neither increase the lipid profile nor decrease weight and BMI.
5. Our results with EE/CPA as individual drug are equal to other different studies in overweight PCOS women with insulin resistance demonstrating an efficient decrease in testosterone, androstenedione, DHEA-S and FAI.
6. Increased triglyceride levels have been conjointly ascertained in this study. The elevated triglyceride levels at the end of the treatment is demonstrated by the common impact of estrogen–progestin formulations on triglyceride.
7. Statistical tests have failed to find out any trend in the inconsistency of insulin levels or its response to OGTT after combining each medication. This outcome in turn may point out that there is no probable interaction between these two drugs.
8. The data in overweight PCOS affected ladies point out that a lower dose of oral contraceptive including EE/CPA does not raise insulin levels compared to results in non-obese PCOS women.
9. The shortage of raise in insulin resistance by the EE/CPA drug utilized in this analysis might be associated with the direct anti-androgenic action of CPA.

10. The data of this study have revealed that rosiglitazone is potent in decreasing insulin in a similar rate irrespective of the presence or absence of EE/CPA.

11. In contrast, rosiglitazone might be more efficient than antidiabetic drug as metformin has been rumored as inefficient in decreasing insulin levels in non-obese PCOS women conjointly taking EE/CPA.

12. Besides this, rosiglitazone may conjointly decrease visceral fat that acts as an important agent in insulin resistance.

2.10 A study to compare the effects of metformin alone and combined with ethinyl estradiol-cyproterone acetate in polycystic ovary syndrome.

Among the dominant symptoms of PCOS, insulin resistance is mostly common and the drug metformin has been widely used to treat this condition. The purpose of this study is to compare the impacts of metformin, a biguanide hypoglycemic agent used independently and in combination with an estrogen-progestin pill ethinyl estradiol (EE)-cyproterone acetate (CA) to manage hyperandrogenism and enhance insulin sensitivity and dyslipidemia. In this prospective clinical analysis, thirty PCOS ladies (age 19-29; mean 23.8 ± 2.8) have been divided into two groups; group 1 have received metformin only and group 2 received metformin along with EE-CA pill at the initial two months of this study. This clinical trial have been lasted for six months. The results of this study have suggested that combined utilization pf metformin and EE-CA pill is more efficient for pathogenic impacts and clinical enhancement of symptoms of hyperandrogenism in states of PCOS. Besides this, the combination treatment has been presumed to be effective in case of patients who are non-responsive to metformin monotherapy (Mitkov et al., 2005).

Assay method:

1. Microparticle enzymatic immunoassay (MEIA) has been used to determine immune reactive insulin.

2. Enzyme-linked immunosorbent assays (ELISA) has been used to determine the levels of testosterone, SHBG and DHEAS.

Result:

Table 10: Comparison between EE/CPA, MET and combined groups.

CRITRIA	EE/CPA	EE/CPA+MET(Group2)	MET (Group 1)
Hormonal Changes	Fall in TT levels.	Fall in hyperinsulinemia, rise in insulin sensitivity, rise in SHBG, FAI.	Inhibit hepatic gluconeogenesis. Increased uptake of glucose by muscles.
BMI	No change.	No change.	No change.
Testosterone levels		Mean difference in testosterone is 0.82(95%). Enhancement of testosteronaemia.	Mean difference in testosterone is 0.45(95%)
SHBG values		More increased.	Less increased.
FAI values		Less significant changes.	Extreme significant changes.

Discussion:

- a) The results of this study precisely implies that intermittent application of EE-CA prompts to quicker reduction in testosterone levels.
- b) The inclusion of EE-CA, a powerful antiandrogenic drug significantly removes the concentration of biologically active testosterone.

- c) Due to the significant reduction of IRI and HOMA-IR estimations, it has been claimed that combination of metformin with EE-CA not only prevents hyperinsulinaemia yet prompts to enhancement of insulin sensitivity by greatly reducing androgens.
- d) Short-term use of metformin does not cause impairment of insulin sensitivity and does not affect the lipid profile.
- e) As there has been no change observed in BMI, WHR and cholesterol values, therefore this combination treatment may be appropriate for applications in patients representing metabolic syndromes.
- f) In case of women who do not wish to be pregnant, the monotherapy with oral contraceptives may be a treatment of choice.
- g) The authors have used the combination treatment for the purpose of avoiding the negative effects of OCPs on body weight, lipid profile and to accelerate the efficacy of metformin in the treatment of hyperandrogenaemia.

2.11 A study on the impacts of metformin and ethinyl estradiol-cyproterone acetate and their combination on endocrine, clinical and metabolic parameters in PCOS women.

It is evident that whatever the cause of PCOS, there will be always a vicious cycle between insulin resistance and hyperandrogenemia that may prevail the reproductive, endocrine and metabolic anomalies generally observed in PCOS affected women. The purpose of this study is to evaluate the endocrine, clinical and metabolic impacts of three treatment procedures:

1. Powerful antiandrogenic drug: ethinyl estradiol-cyproterone acetate (Group A)
2. A hypoglycemic biguanide drug: Metformin. (Group B)

3. Combination of metformin and ethinyl estradiol-cyproterone acetate. (Group C)

60 PCOS affected women (age between 19-35 years) have been divided into three above groups among those 25 are obese (BMI > 25 kg/m²) and 35 are non-obese (BMI < 25 kg/m²). This prospective randomized trial has been continued for three months. The results of this study have demonstrated that combined treatment modality is more efficient in eradicating hyperandrogenemia of both obese and non-obese PCOS women compared to metformin as well as may decrease insulin concentrations to a greater extent than EE-CA pill. Therefore, combined treatment procedure is considered as more efficient therapeutic choice for both obese and non-obese PCOS affected women (Wu, Zhu, Jiang, & Cao, 2008).

Assay method:

1. Chemiluminescence assay has been used to determine the blood levels of testosterone, FSH and LH.
2. Glucose oxidase method has been used to determine blood sugar levels.
3. Radioimmunoassay has been utilized in the determination of insulin levels.

Result:

Table 11: Comparison between Groups A, B and C.

Criteria	Group A		Group B		Group C	
BMI	Insignificant rise both in obese and non-obese women.		Obese women: remarkably reduced (from 25.6±0.6 to 22.4±0.8kg/m ²)	Non-obese women: No significant changes (from 21.5±1.8 to 20.9±1.4 kg/m ²).	No Changes.	
2. Mean WHR	Insignificant changes.		Reduced notably (from 0.83±0.02 to 0.78±0.03)	No significant changes (from 0.78±0.03 to 0.77±0.04)	Insignificant changes.	
3. FG score	Obese women: Significant reduction from (8.3 ± 1.0 to 6.8 ± 1.3)	Non-obese women: Significant reduction (from 7.8±1.3 to 6.9±1.1)	No significant changes in non-obese women		Obese women: Significant reduction (from 8.2±1.4 to 6.3±0.8)	Non-obese women: Significant reduction (from 7.8±2.1 to 6.7±1.2)
4. Menstrual regularity	Restored.		Restored only in 28% of participants.		Restored.	
5. Hormone concentration	Reduced significantly.		Significant reduction in testosterone levels in obese women (from 3.3±0.7 to 2.7±0.6 nmol/l, p<0.05) and non-obese women (from 2.7±0.6 to 2.1±0.5 nmol/l, p<0.05).		Reduced significantly.	
	The levels of LH (obese and non-obese, p<0.01),				the levels of LH (obese and non-obese, p<0.01),	
	testosterone (obese non-obese, p<0.01)				testosterone (obese, p<0.05; non-obese, p<0.01) LH/FSH ratio	

Table 11: Comparison between groups A, B and C.

6. Fasting insulin levels	p >0.05.	Remarkably decreased from 20.6±4.5 to 14.5±4.1 mIU/l, p<0.01 (obese); from 14.8±6.0 to 11.2±4.2 mIU/l, p <0.05 (non-obese).	Remarkably decreased from 19.2±5.6 to 15.1±4.2 mIU/l, p<0.05 (obese); from 13.8±5.1 to 10.8±3.6 mIU/l, p<0.05 (non-obese)
7. Glucose: insulin ratio	Insignificant change (p>0.05).	Remarkably raised (obese: from 4.9±2.0 to 6.5±1.4, p<0.05; non-obese: from 4.7±1.4 to 5.8±0.8, p<0.05)	Insignificant change (p>0.05).

Discussion:

A. Ethinyl estradiol-cyproterone acetate:

1. Beneficial for preventing menstrual irregularities and hirsutism in overweight and lean PCOS women.
2. Significant decrease in LH, LH: FSH ratio and testosterone concentrations.
3. Though oral contraceptives have been accused for increasing body weight, insulin resistance and insulin concentration, this present study did not observe any remarkable alterations in these factors.
4. It remarkably enhances the hyperandrogenemia in overweight and lean PCOS women beyond deteriorating insulin sensitivity.

B. Metformin:

1. Noticeably enhances BMI in overweight PCOS women whereas no impact on lean women.

2. Enhancement of insulin resistance and decrease in insulin and testosterone concentrations in both overweight and lean women.

C. Combination of EE-CA and MET:

1. Remarkable enhancement of hyperinsulinemia in overweight and lean PCOS women along with a noticeable reduction of testosterone, LH and LH: FSH ratio.

2. Restoration of menstrual cycle and enhancement of hirsutism score in all participants.

3. More efficient in enhancing hyperinsulinemia and hyperandrogenism of PCOS women as few adverse impacts of oral contraceptives might be decreased by the inclusion of metformin.

2.12 A study on the impacts of metformin and ethinyl estradiol–cyproterone acetate on lipid profile in both overweight and lean women with polycystic ovary syndrome.

PCOS women with atherogenic lipid profile are at greater risk of cardiovascular diseases. The present study aims to find out the impacts of metformin and ethinyl estradiol-cyproterone acetate on lipid profile of both overweight and lean PCOS affected women. 35 PCOS women (18 obese and 17 non-obese; (mean age, 29.6 ± 1.1 years; mean BMI, 35.1 ± 1.2 kg/m²; mean age, 28.2 ± 1.2 years; means \pm S.E. throughout) have been assigned to six months treatments with metformin or ethinyl estradiol–cyproterone acetate oral contraceptive pills. The results of this study have suggested that metformin might be efficient to prevent the risks of cardiovascular diseases in PCOS ladies with insulin resistance. Besides this, ethinyl estradiol-cyproterone acetate drug is also beneficial to restore menstrual cycle and to treat hirsutism (Rautio, Tapanainen, Ruukonen, & Morin-Papunen, 2005).

Result:

Table 12: Comparison between MET, and EE-CA treated groups.

Criteria	MET	EE-CA
1. Mean BMI and WHR	Noticeably reduced.	
2. Serum levels of HDL cholesterol	Raised.	
3. Cholesterol: HDL ratio	Notably reduced.	Reduced.
4. Serum triglyceride levels	Reduced.	Remarkably raised.
5. Serum levels of total cholesterol and HDL		Remarkably raised.
6. Systolic and diastolic blood pressure	Fall. Systolic (from 126 ± 3.6 to 117 ± 2.8 mmHg, $P=0.02$) and diastolic (from 81 ± 2.0 to 78 ± 2.0 mmHg, $P=0.05$)	No change.

- I. In the obese group, total C: HDL-C ratio reduced remarkably in both MET and EE-CA groups.
- II. Serum HDL cholesterol level noticeably raised in both the MET (from 1.1 ± 0.1 to 1.4 ± 0.1 mmol/l $P<0.005$) at three and six months and the EE-CA group (from 1.0 ± 0.1 to 1.3 ± 0.1 mmol/l; $P<0.001$) at three and six months.
- III. Serum triglyceride levels have not been notably changed in the MET group, yet have raised in EE-CA treatment (from 1.5 ± 0.1 to 2.0 ± 0.2 mmol/l ($P<0.05$) at three months and to 1.9 ± 0.1 mmol/l ($P<0.01$) at six months.

- IV. In lean women group, serum total cholesterol concentration have raised remarkably in the OC group.
- V. Serum HDL cholesterol level raised from 1.4 ± 0.1 to 1.7 ± 0.1 mmol/l at 3 months ($P<0.01$) and to 1.8 ± 0.1 mmol/l at 6 months ($P<0.001$) in the OC group.

Discussion:

- a) The abnormal lipid profile observed in PCOS affected women have indicated that obesity (abdominal fatness) and insulin resistance are the major determinants of lipid and metabolic abnormalities in PCOS women.
- b) Metformin has been found to enhance lipid values precisely by raising serum HDL cholesterol levels along with a mild to moderate decrease in blood pressure. Therefore, metformin by virtue of its advantageous impact on lipid profile and raised blood pressure, might be necessary in preventing cardiovascular diseases, mainly in overweight women.
- c) With increased dose of metformin, side-effects become more prevalent. Therefore it necessitates the management of metformin dose to keep within 1000mg/day.
- d) In the metformin treated group, the enhancement of obesity principally the abdominal obesity has been observed with a concurrent reduced release of free fatty acids (FFAs) from adipose tissue.
- e) The negligible influence of estrogen of the EE-CA pill might be responsible for few of the advantageous impacts of EE-CA therapy on lipid levels.
- f) The inclusion of metformin in EE-CA drug has demonstrated that it may enhance the lipid profile and insulin sensitivity in lean patients affected with PCOS.

2.13 A study on the correlation of impacts of 3 mg drospirenone in addition to 20 mg ethinyl estradiol alone or joined with metformin or cyproterone acetate on classic metabolic cardiovascular risk factors in non-obese women with polycystic ovary syndrome.

Drospirenone is an extraordinary progestin, being identified with 17α -spironolactone instead of derived from 19-nortestosterone. It shows both antimineralcorticoid and antiandrogenic property. The antiandrogenic action may clarify the impact on insulin sensitivity. In any case, the antimineralcorticoid property might be associated with this impact. Truth be told, aldosterone instigates insulin resistance by a restraint of the biosynthesis what's more, affinity of insulin receptors. Forty-eight hirsute consecutive patients (mean age was 24 years; range, 15–34 years) with PCOS have been randomized to receive 3 mg drospirenone in addition to 20 mg ethinyl estradiol alone or in addition with metformin or cyproterone acetate and is assessed after six months. Taking everything into account, the consequences of the present investigation do not reinforce a harmful impact of all OCs in patients with PCOS. Specifically the present information demonstrate that in lean PCOS women, DRP along with 20 mg EE improves the metabolic profile of these patients— an impact that has not been additionally improved with metformin supplementation. On the other hand, the inclusion of high portions of CPA decays insulin and glucose metabolsim, making its utilization troublesome in hirsute patients with PCOS (Fruzzetti et al., 2010).

Assay method:

1. An enzymatic technique has been used to determine serum levels of total cholesterol, HDL cholesterol, and triglycerides.
2. An immunoradiometric technique has been used to measure the levels of insulin.

3. Glucose oxidase technique has been used to determine blood sugar levels.

Result:

Table 13: Comparison between DRP/20EE, MET and CPA combined treated groups.

Criteria	DRP/20EE	DRP/20EE+MET	DRP/20EE+CPA
BMI/BP	-	-	-
Lipid profile	-	Rise in HDL-C.	Rise in TCL, total C significantly higher.
AUC for insulin	Fall.	Fall.	Rise.
Indexes of insulin sensitivity	Improved.	Improved.	Worsened.
HOMA-IR index	Slightly decreased.	Slightly decreased.	Significantly increased.
Glucose: insulin index	Significantly increased.	No change.	Significantly decreased.
Fasting and stimulated glucose levels	No change.	No change.	Significantly increased.
Fasting insulin levels	No change.	No change.	Significantly increased.

Discussion:

- a. The present outcomes demonstrate that DRP added to 20 µg EE is considered to be secure in lean hirsute PCOS women, demonstrating by and large advantageous impacts on the metabolic profile.

- b. The most fascinating finding is the absence of a negative effect on carbohydrate metabolism. On the other hand, a development in insulin sensitivity has been found, as reported by the reduction of basal insulin and of the insulin response to an oral glucose load, prompting an enhancement of the index of insulin sensitivity.
- c. No diminishing effect in lipid metabolism have been found.
- d. Prior studies have found in fifteen hirsute PCOS women receiving DRP (3 mg) added to a higher portion of EE (e.g., 30 µg) for twelve menstrual cycles demonstrated that there has been no adverse effect on glycoinsulinemic homeostasis and a pattern toward a development of lipid profile. These outcomes propose that the utilization of DRP can lessen the metabolic problems that are explicit to women with PCOS, in regards to the conceivable utilization of OCs in these patients.
- e. OCPs with 3rd generation progestins like desogestrel, gestodene and norgestimate have been thought as neutral yet it has been found to reduce insulin sensitivity in some cases.
- f. The androgenicity of every one of the 19 nortestosterone subsidiaries may assume an activity in diminishing insulin sensitivity.
- g. Drospirenone is the main progestin with aldosterone antagonist activity. Based on these information, it could be guessed that the development of insulin sensitivity found in this investigation may be credited at any rate to some degree to the DRP–aldosterone antagonist activity fundamentally.
- h. In addition, DRP when added to ethinyl estradiol has become capable to reduce hirsutism and testosterone concentrations and more importantly raising SHBG concentrations in PCOS women.

2.14 A double-blind, placebo-controlled study to assess the metabolic and endocrine impacts of rosiglitazone in obese PCOS women.

This is the first assessment demonstrating mechanism of action of rosiglitazone in PCOS affected women utilizing calorimetry and clamp method, the gold standard for the estimation of insulin sensitivity. Thirty obese PCOS affected women (BMI > 25 kg/m², mean age 29.1 ± 1.2 years) have been randomized to receive rosiglitazone or placebo and this medical investigation has been continued for four months. Treatment with rosiglitazone have effectuated a noticeable development in insulin resistance, menstrual cycle regularity, hyperinsulinaemia and to a lesser degree hyperandrogenism in obese PCOS ladies (Rautio et al., 2006).

Assay method:

1. Fluoroimmunoassays have been used to determine the levels of sex hormone-binding globulin (SHBG), LH and FSH.
2. The levels of dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), androstenedione and 17-hydroxyprogesterone (17-OHP) have been determined by using radioimmunoassays.
3. Immunoenzymometric method has been used to determine the levels of human serum insulin-like growth factor-binding protein-1 (IGFBP-1).
4. An automatized chemiluminescence method has been used to determine the levels of testosterone, insulin and C-peptide.

Result:

Table 14: Comparison between ROSI and PLA treated groups.

Criteria	ROSI	PLA
1. BMI	Significant rise (P=0.006).	
2. Menstrual cycle	More regular (P= 0.005).	
3. Serum SHBG conc. and Serum conc. of androstenedione, 17-OHP	Significant rise (P= 0.04).	
4. Serum DHEA and DHEA-S	Significant rise (P=0.01) and (P= 0.05).	
5. Fasting plasma glucose levels	Significantly decreased (P=0.03).	More than ROSI group.
6. AUCglucose during OGTT	Significant rise (P=0.002).	Significant worsening(P=0.05)
7. Fasting C-peptide conc.	Significantly decreased (P=0.01).	
8. AUCinsulin	Significantly reduced (P=0.004).	
9. M value	Raised (from 33.4 ± 3.27 to 40.0 ± 5.51 $\mu\text{mol/kg min}$, P = 0.04).	
10. Rate of Glucose oxidation	Raised.	Less than ROSI group (P=0.05).
11. Lipid oxidation rates	Reduced (P=0.09)	

Discussion:

1. Though there has been no reduction in fasting serum insulin concentrations, the enhancement of insulin resistance has been accompanied by a reduction in hyperinsulinaemia represented by an enhancement in AUC_{insulin} at the time of oral glucose tolerance test.
2. Through developing insulin sensitivity and increasing insulin impacts at peripheral target points like adipose tissue and skeletal muscle beyond any straight impact on pancreatic insulin secretion, rosiglitazone has been found to enhance glycemic control.
3. The rise in M value that means the peripheral insulin sensitivity has been accompanied by the increased rate of glucose oxidation and non-oxidation with rosiglitazone treatment.
4. In this study, rosiglitazone has reduced serum free fatty acid levels through the increment of free fatty acid uptake and oxidation in skeletal muscle. However this impact prompts to reduce competition between the low levels of circulating free fatty acid and glucose, hence enhancing insulin sensitivity.
5. The thiazolidinedione group's drug rosiglitazone is an insulin sensitizing agent without any stimulatory impact on pancreatic secretion, yet it shows a β -cell-sparing impact in diabetic patients suffering with impaired β -cell function.

2.15 A study on the endocrine and metabolic impacts of rosiglitazone in lean PCOS women.

In order to exert a higher risk for the progress of early onset of type 2 diabetes mellitus, the insulin resistance functions adjointly with changes in β -cell function. Hyperandrogenism-associated anovulation along with its result, lack of progesterone, induces endometrial hyperplasia and raises the hazards for endometrial cancer. The insulin sensitizing group of drugs under thiazolidinedione class, induces significant dose-dependent enhancement in muscle

insulin sensitivity. Rosiglitazone is an ideal insulin sensitizer of this class beyond any complains of severe adverse impacts. It has been anticipated by the authors of this study that rosiglitazone might exhibit a dose-dependent enhancement in carbohydrate metabolism, ovulation irregularities, and hirsutism of PCOS women. 40 PCOS affected ladies and with impaired glucose tolerance test (IGT) have been constantly placed to the eight-month time period with rosiglitazone at either 2 mg/day or 4 mg/day. The mean age is 31.4 ± 0.9 and 29.4 ± 1.7 years and the BMI is 24.2 ± 1.3 kg/m² and 23.9 ± 1.9 kg/m² in the rosiglitazone 4mg group and rosiglitazone 2mg groups accordingly. This study have found these following outcomes:

1. Development of glucose intolerance,
2. Enhancement of insulin resistance,
3. Reduction of total and free testosterone levels,
4. Enhancement in endogenous ovulatory activity (Dereli, Dereli, Bayraktar, Ozgen, & Yilmaz, 2005).

Assay method:

1. Chemiluminescent enzymatic immunoassay method has been used to determine the blood levels of FSH, E2, LH, progesterone, prolactin and cortisol.
2. Standardized radioimmunoassays have been used to determine the blood levels of DHEA-S, 17-OH progesterone, free testosterone, free T4 and TSH.
3. Glucose oxidase method has been used to determine blood sugar levels.

Result:

A. Changes during 3rd month:

Table 15: Comparison between ROSI (4mg) and ROSI (2mg) treated groups.

Criteria	ROSI(4mg)	ROSI (2mg)
1. Body weight	Rise.	Rise.
2. 2 nd hour glucose levels	118.8± 10.3	129.3± 9.3
3. Free testosterone levels	Greatly reduced.	Less reduction.
4.The interval between menstrual cycle	Significantly reduced.	Significantly reduced.
5. Ovulation	6 women.	4 women.
6.Side-effects and liver enzymes	No side-effects with normal limits of liver enzymes.	No side-effects with normal limits of liver enzymes.

B. Changes during final visit:

Table 16: Comparison between ROSI (4mg) and ROSI (2mg) treated groups.

Criteria	ROSI(4mg)	ROSI (2mg)
1. Body weight/ BMI	Greatly raised.	Greatly raised.
2. The HDL levels	Raised.	Raised.
3. Triglyceride levels	Reduced.	Reduced.
4. Free testosterone levels	Greatly reduced.	Less reduction.
5.The interval between menstrual cycle	Significantly reduced.	Significantly reduced.
6. Ovulation	17 women.	14 women.
7. Normal glucose tolerance test	2 women.	1 women.
8.Side-effects and liver enzymes	No side-effects with normal limits of liver enzymes.	No side-effects with normal limits of liver enzymes.

Discussion:

1. In spite of increase in BMI, there has been significant dose-related enhancement of insulin sensitivity.
2. Rosiglitazone has helped to normalize the glucose tolerances of nineteen subjects of 4mg group (95%) and fifteen subjects in 2mg group (75%).
3. Rosiglitazone has been significantly effective in reducing excess levels of free testosterone.
4. Rosiglitazone has been also effective in reducing Ferriman-Gallwey score and to reduce hirsutism in a dose-related manner.
5. The noticeable dose-related increase in estradiol and reduction in estrone levels, exhibiting matured follicles has been also found by this study.
6. There has been equal yet more important decrease in hba1c levels.
7. Greater ovulation rates have been reported by this study.
8. In addition, most of the effects exerted by rosiglitazone has been found in the 4mg dose group, therefore it indicates that this dose of rosiglitazone must be administered to obtain remarkable development in insulin sensitivity not depending on weight reduction, significant reduction in the best biochemical indicator of hyperandrogenism, reducing androgen levels and improving ovarian dysfunction.

2.16 A study to compare the effects of metformin and pioglitazone on serum androgen and insulin resistance in overweight, insulin-resistant PCOS affected women.

From the year of 1980, insulin resistance and hyperinsulinemia has been robustly related to PCOS and has become mostly but not extraordinarily prevalent in overweight women. The presence of insulin resistance within PCOS women depends on the basis of assay method utilized and the population of women analyzed. Metformin has been widely used for decades in the treatment of type II diabetes mellitus. It is a biguanide antihyperglycemic drug. Patients who do not give response to metformin therapy, pioglitazone, a member of thiazolidinedione group has been applied to treat the problems associated with insulin resistance in PCOS women. 52 PCOS women have been randomly assigned to either pioglitazone (30 mg/day n =25) or metformin (850 mg three times daily, n =27) and have been analyzed before and after six months. The outcome of this study has recommended that the effectiveness of pioglitazone closely resembles metformin in terms of development of insulin sensitivity and hyperandrogenism irrespective of an increment in body weight, body mass index along with waist-to-hip ratio related to pioglitazone (Ortega-González et al., 2005).

Assay methods:

1. The levels of FSH, LH, PRL and TSH have been determined by immunoradiometric kits.
2. The levels of rest of the hormones have been determined by RIA kits.
3. Serum glucose levels have been determined through the glucose oxidase technique utilizing an automatic enzymatic auto analyzer.
4. Serum TC, ALT, LDL, TG, AST, HDL along with alkaline phosphatase have been estimated by an automatized spectrophotometer.

Result:

Table 17: Comparison between metformin and pioglitazone.

Criteria	Pioglitazone	Metformin
1. Body weight and Body mass index	Body weight raised by 4.7kg and BMI also raised.	Insignificant rise.
2. Waist-to-hip ratio	Significantly raised (P=0.02).	No change.
3. Ferriman-Gallwey score	Reduced.	Reduced.
4. Fasting serum insulin levels	Significantly reduced (P<0.001).	Reduced (P<0.001).
5. AUCinsulin during 2hours oral glucose tolerance test	Significantly reduced (P<0.002).	Reduced (P<0.05).
6. HOMA-IR index	Significantly reduced (P<0.001).	Reduced (P<0.001).
7. QUICKI	Rise (P<0.001).	Significantly raised (P<0.008).
8. Fasting glucose: insulin ratio	Rise (P<0.001).	Significantly raised (P<0.001).
9. Abortion rates	3/8.	1/8.
10. Gestational diabetes acquired	2/8.	1/8.

Table 18: Comparison between pioglitazone and metformin treated groups.

Parameters	Pioglitazone		Metformin	
	Baseline (n = 17)	6 Months (n= 17)	Baseline (n= 18)	6Months (n=18)
Fasting glucose (mg/dl)	92.5 ± 2.6	88.6 ±1.8	93.4 ±2.9	88.7 ±2.1
AUC-glucose (mg/dl min)	16.383± 742	14.87±1.734	17.337 ±716	14.213 ± 684
Fasting insulin (U/ml)	31.1 ± 1.1	11.1 ± 1.4	31.1 ±1.5	11.0 ± 1.4
AUC- insulin(U/mlmin)	14.884±1.800	10.930 ± 1.24	16.027±2.078	13.415 ±1.830
HOMA-IR	7.03 ± 0.28	2.42 ±0.31	7.21 ± 0.52	2.43 ±0.3
QUICKI	0.29 ±0.001	0.34 ± 0.004	0.29 ± 0.001	0.34 ± 0.003
Fasting G/I ratio	3.02 ± 0.13	9.38 ±1.12	3.19 ± 0.13	9.33 ±1.32
Serum TG (mg/dl)	158.6 ± 14.8	143.7 ±14.4	151.4 ± 11.0	124.6 ±8.96
TC (mg/dl)	189.4 ± 7.7	174.3 ± 7.6	183.1 ±6.3	181.3 ± 7.3
HDL-cholesterol (mg/dl)	41.1±2.1	46.9 ±3.0	38.7 ±2.5	42.6 ±1.9
LDL-cholesterol (mg/dl)	120.5± 6.2	116.1 ± 5.8	111.3 ± 5.6	124.3 ±6.3

Discussion:

1. Both pioglitazone and metformin has been effective in reducing serum levels of free testosterone, androstenedione and reduce hirsutism.

2. Both of the drugs induce equal developments in hormonal and clinical hyperandrogenaemia. This significant development has been happened irrespective of raise in body weight, body mass index, waist-to-hip ratio related to pioglitazone not metformin.
3. This ironical outcome may be attributed to the advantageous conversion from abdominal fat into subcutaneous fat and the subsequent development in insulin sensitivity initiated by thiazolinediones.
4. It has been found that the mechanism by which pioglitazone may enhance hyperandrogenism is nearly analogous to troglitazone's mechanism of action.
5. Pioglitazone has been categorized as pregnancy category c drugs due to its tendency to cause growth hindrance in middle to latter gestation in animal subjects. As a result, pregnancy problems like abortions and development of gestational diabetes has been found more recurrently in the pioglitazone group than metformin.
6. On the contrary, metformin has been considered as pregnancy category b drugs and has been found to be more secured in women who wish to become pregnant or being pregnant.
7. Furthermore, there has been no complains about hepatotoxicity and major side-effects of pioglitazone among the participants of this study.
8. Based on the results of this study, it has been found that pioglitazone has more effectiveness in developing insulin sensitivity as fasting serum insulin levels and AUCinsulin has been remarkably lesser with pioglitazone than with metformin. This result resembles with findings in patients with type ii diabetes mellitus.
9. In this study, a maximal dose (2.5g/day) of metformin and a submaximal dose (30mg/day; maximal recommended dose is 45mg/day) has been used which leads to this desirable effects on insulin sensitivity.

2.17 Differential impacts of metformin along with clomiphene citrate in infertile lean PCOS women to induce ovulation.

Among the diagnosed symptoms of PCOS, infertility problems with ovulatory dysfunction has been more prevalent among PCOS affected reproductive women. Holtkamp et al. had applied clomiphene citrate for the first time to induce ovulation in women suffering with oligomenorrhea. Among the anti-estrogenic and estrogenic functionality, clomiphene citrate has been found to act significantly by blocking estrogen's action, resulting in an increment of pulse frequency along with FSH and LH levels and raising the follicles of the ovaries thus inducing ovulation. Moreover, this hormonal drug is available at lower price, can be administered without any critical observation and shows few dose-related side-effects. This clinical trial has selected 100 PCOS women and assigned them into either clomiphene citrate or metformin (Group A: metformin+ placebo and Group B: placebo+ clomiphene citrate; each with fifty women) and this study has been continued for six months. The results of this randomized clinical investigation have explained that to enhance ovulatory function metformin is more efficacious compared to clomiphene citrate in lean barren PCOS affected women (Palomba et al., 2005)..

Assay method:

1. Distinct RIA has been used to determine plasma hormone levels.
2. An immunoradiometric technique has been used to estimate SHBG concentrations.
3. A solid-phase chemiluminescent enzymatic immunoassay method has been utilized to determine serum insulin concentrations.
4. Glucose oxidase technique has been used to measure blood glucose concentrations.

Result:

Table 19: Differences between Group A and Group B.

Criteria	Group A	Group B
1. Rate of pregnancy per ovulation cycle	Remarkably greater 15.1%	Lesser than group A 7.2%.
2. Rate of miscarriage	9.7%	37.5%
3. Rate of live-birth	83.9%	56.3%

Discussion:

1. The administration of gonadotropins to induce ovulation requires careful monitoring and skilled operator as well as it is too costly and needs a lot of time investment.
2. Therefore, treatment with clomiphene citrate has become popular to induce monotherapy as well as to escape from multiple pregnancies related with multiple follicular development in cases of PCOS.
3. Patients who are resistant to clomiphene citrate, metformin may be a cost-effective choice of treatment compared to laparoscopic ovarian diathermy.
4. Besides this, metformin may show its function on reproductive system by inducing ovulation and increasing the rate of pregnancy.
5. The inclusion of metformin into clomiphene citrate exhibits 3.5 times more effective activity compared to clomiphene citrate alone.
6. As PCOS affected women have greater hazards of abortion, treatment with metformin shows lesser abortion rates compared to the clomiphene citrate group.

7. The advantageous impact of metformin on pregnancy has been attributed to the fact that metformin acts on the oocytes or endometrium or embryos.

2.18 A study on the comparative effects of clomiphene citrate or letrozole to induce ovulation in unproductive PCOS affected women.

The underlying reason behind the infertility problem of PCOS affected women is anovulation. This study has aimed to differentiate between clomiphene citrate and letrozole as first-line ovulation initiation medication in PCOS affected women who are unable to become pregnant. One hundred and three PCOS women who are ingenuous to treatment has been randomized to take either clomiphene citrate (n=52) or letrozole (n=51) and Human Chorionic Gonadotropin (HCG) injection has been given to all participants. The data of this study has demonstrated the fact that letrozole is more effective in inducing greater mono-follicular growth and increased rate of pregnancy than clomiphene citrate when used as first-line ovulation initiation medication. This improved effects of letrozole might be associated with ethnic variations in PCOS women (Kar, 2012).

Assay method:

1. Changes in follicular growth has been screened by transvaginal sonography.
2. Measurement of cervical mucus has been done on the HCG injection time by following cervical mucus scoring standards.
3. Sonographical results and progesterone level at 21st day has been used to assure ovulation rates.
4. Cardiac activity has been visualized by TVS to monitor ongoing pregnancy.

Result:

Table 20: Comparison between clomiphene citrate and letrozole treated groups.

Criteria	Clomiphene citrate	Letrozole
1. Multiple follicular growth rates	45.16%	20.51%
2. Rate of ovulation	60.78%	73.08%
3. Endometrial thickness (mm)	7.61 ± 1.96	7.65 ± 2.10
4. Pregnancy occurrences	4/51 (7.84%)	11/52 (21.56%)

Discussion:

1. The mean endometrial thickness has been marginally greater in the letrozole group than clomiphene citrate group.
2. Rate of pregnancy has been also greater in the letrozole group.
3. This greater pregnancy rate has been attributed to the fact that most of the Indian women are insulin resistant and exhibit central visceral fatness that exposes them to clomiphene citrate resistance.
4. Hyperinsulinemia, the major diagnostic factor of PCOS has greatly influenced the development of clomiphene citrate resistance in PCOS women.
5. The induction of ovulation by letrozole has been greater than clomiphene citrate and it has been attributed greatly to the mechanism of action of letrozole.
6. Letrozole mainly acts by blocking the transformation of androgens into estrogens, thereby secreting FSH from pituitary as well as increasing follicular response to FSH by amplifying FSH receptor gene expression.

2.19 Comparison of metformin and rosiglitazone (individually/combined) on endometrial histology and ovarian hormone synthesis in PCOS women.

The PCOS affected women has been concurrently found to have high insulin levels and excess androgen concentrations which leads to various problems like development of type II diabetes mellitus, anovulatory infertility, hirsutism and so on. The utilization of thiazolidinediones like rosiglitazone, pioglitazone, troglitazone has been carried out to reduce the earlier clinical manifestations associated with hyperinsulinemia. This present study have selected sixteen PCOS women and assigned them to either rosiglitazone (n=9) or metformin (n=6) and continued for six months (three months for individual therapy and other three months for combination therapy). The findings of this study have demonstrated that insulin sensitizers like metformin or rosiglitazone may show advantageous impacts on endometrium by increasing follicular growth. Additionally, rosiglitazone has been found to be more effective than metformin on increased insulin and androgen concentrations in overweight PCOS women. However, combination therapy has no favorable impacts compared to individual therapy (Legro et al., 2007).

Assay method:

1. Plasma glucose concentrations have been estimated by glucose oxidase method.
2. Double antibody radioimmunoassay has been used to estimate insulin concentrations.
3. Competitive enzyme immunoassays (EIAs) have been utilized to estimate main urine metabolites like estrone-3-glucuronide (E1G) and pregnanediol-3-glucuronide (PdG).
4. A microtiter plate spectrophotometer has been used to quantify color signal in every assay methods.

Result:

Table 21: Comparison between rosiglitazone and metformin effects alone or in combination therapy.

Criteria	ROSI	MET	ROSI+MET
1. Free and weakly-bound testosterone	Noticeable reduction.		
2. Insulin and glucose estimations after OGTT testing	Remarkable development.		
3. AUC PdG/E1G urinary proportion	More than metformin	Less than rosiglitazone.	
4. Body weight			Remarkable increase in body weight.
5. Blood pressure			Remarkably raised.

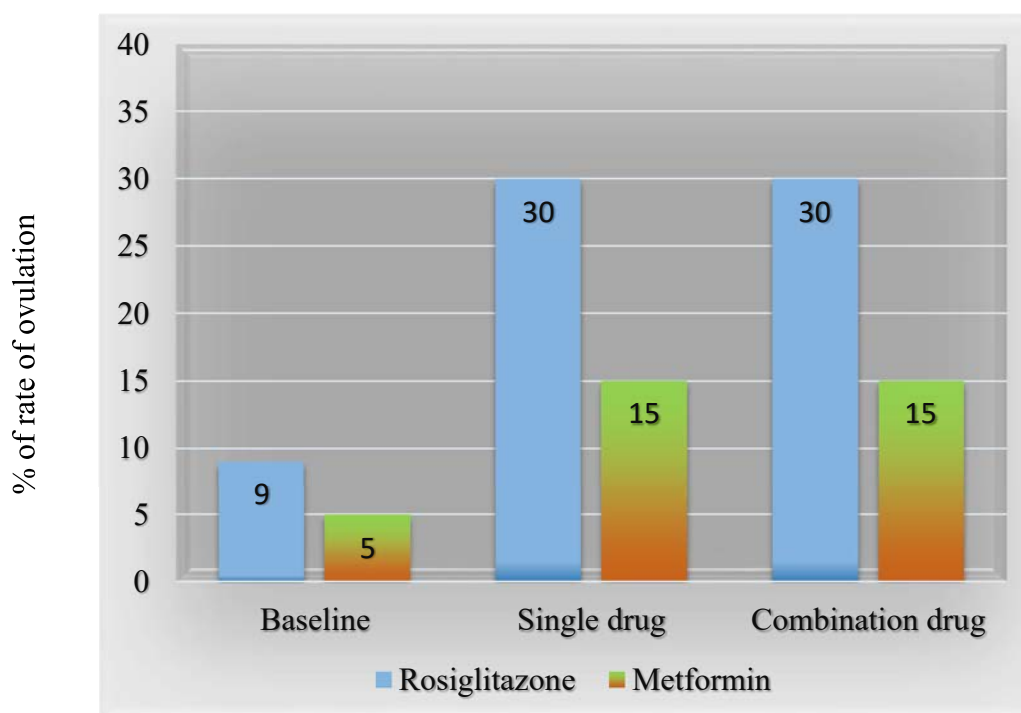


Figure 19: Rate of ovulation by study phase and by rosiglitazone and metformin.

Discussion:

1. Noticeable development of the free and weakly-bound testosterone, in urine progestin/estrogen proportion and combined insulin and glucose levels after oral glucose tolerance test by rosiglitazone than metformin.
2. The rate of ovulation has been increased by both metformin and rosiglitazone.
3. Rosiglitazone has been more effective in decreasing circulating testosterone levels and insulin levels as well.
4. Clinical trials on metformin has been resulted with increased uterine blood circulation.

2.20 Impacts of metformin in PCOS women with or without insulin resistance.

Metformin has numerous benefits on PCOS affected women both with insulin resistance and without insulin resistance. This present study has selected forty six PCOS women (age: 19-33 years) and has been allocated to metformin therapy along with gestagens, dydrogesterone, micronized progesterone. The participants have been divided into two groups: insulin resistance and without insulin resistance. This study has been continued for three months (Nawrocka & Starczewski, 2007).

Assay method:

1. Immunoenzymatic technique has been utilized to measure prolactin, adrostenedione, insulin, LH, FSH and SHBG levels.
2. A microparticle enzyme immunoassay has been used to estimate testosterone levels.
3. Plasma glucose levels have been determined by glucose hexokinase technique.

Result:

Table 22: Changes in overall groups before and after treated with metformin.

Criteria	Before treatment	After treatment
1. BMI	27.40 kg/m ²	Reduced (26.69 kg/m ²).
2. LH and FAI levels		Remarkably reduced.
3. FSH: LH		Remarkably raised.
4. SHBG levels		Remarkably raised.
5. Fasting insulin levels (Miu/ml)	12.77	Reduced (9.89)
6. HOMA-IR	5.91	Remarkably reduced (3.46).
7. Ferriman-Galwey score	≥8	<8 (reduced).

8. A remarkable decline in BMI has been found in the group of women who are not insulin-resistant.

9. SHBG levels also have greatly increased in non-insulin-resistant women.

Discussion:

1. There has been noticeable reduction of insulin levels only in the groups with insulin resistance; changes of insulin levels in non-insulin-resistant women has been insignificant.

2. In the groups of non-insulin-resistant women, SHBG levels have raised more significantly.

3. The changes in SHBG levels has been attributed to the reduction of BMI as alteration in hepatic metabolism leads to reduced fat metabolism and increased protein production like SHBG.

4. FAI levels has been reduced in women without insulin resistance.

5. It has been recommended by this present study that if metformin does not work in infertile women, clomiphene citrate may be incorporated in the treatment method to induce ovulation.

6. The reduction of HOMA-IR in insulin-resistant subject's group has indicated that metformin may improve insulin sensitivity in PCOS women.

7. Metformin has been resulted with a decrease in BMI in both insulin resistant and non-insulin-resistant women, it has no impacts on waist-to-hip ratio.

2.21 Differentiation of the impacts of clomiphene citrate-estradiol valerate and letrozole on thickness of the endometrium, rate of pregnancy and abortion in unproductive PCOS women.

Consideration of the thickness of the human endometrium has been more significant in treat infertility problems. If the thickness of the endometrium is lower than 6-8mm, rate of pregnancy may be reduced as well. Therefore, the drugs which leads to enhancement of endometrial thickness have attracted the most attention from prospective researchers. In fact, increased endometrial thickness has been found to promote the replacement of the embryo, thereby decreasing hazards of continuous abortion as well as raises the rate of pregnancy. Moreover, the inclusion of ethinyl estradiol into clomiphene citrate may induce a beneficial response of the endometrium in unproductive PCOS affected women. In this present study, one hundred PCOS women have been divided into two groups: Group A (clomiphene citrate+estradiol valerate) and group B (letrozole+placebo). Mean age in group A has been 30.34 and in group B 29.62. Persistence of infertility in group A and B has been 3.37 and 3.85 years respectively. The results of this study supports the fact that letrozole has improved endometrial thickness and rate of pregnancy in unproductive PCOS women (Seyedoshohadaei et al., 2016).

Assay method:

1. By transvaginal sonography, the thickness of the endometrium has been determined.
2. To blind this investigation, an operator of infertility has undertaken the transvaginal sonography.
3. Drugs has been prescribes by a gynecologist.

Results:

Table 23: Comparison between Group A and Group B.

Criteria	Group A		Group B.	
	Before treatment	After treatment	Before treatment	After treatment
1.Thickness of the endometrium (mm)	5.34	7.26	5.68	8.17
2. Total pregnancy numbers	16%		More (32%).	
3. Rate of abortion	No abortion.		Five cases of abortion.	
4. Total live-birth numbers	Lesser.		Higher.	

Discussion:

1. The thickness of the endometrium has been significantly greater in the letrozole compared to the clomiphene+estradiol valerate treated group.
2. The rate of pregnancy has increased as two folds in the letrozole group than clomiphene+estradiol valerate group and this finding is consistent with the results of the trial by Seyedoshohadaei et al.

Chapter 3

Conclusion

Metformin may be beneficial in decreasing the hazards of cardiovascular diseases in PCOS women, to induce ovulation efficiently, to reduce hyperinsulinemia and hyperandrogenism. Nevertheless, the combined treatment with metformin plus ethinyl estradiol-cyproterone acetate has better outcome than the individual treatment of each drug. Metformin has been found to be superior to clomiphene citrate as metformin has shown a significant increment in the rate of pregnancy in lean PCOS affected women. Another thiazolidinedione groups of drug rosiglitazone has been found to enhance insulin sensitivity, induce ovulation, reduce excess androgen levels and cure hirsutism in a dose-dependent manner. To differentiate between insulin sensitizing drugs and oral contraceptives like estradiol-cyproterone acetate it has been demonstrated that estradiol-cyproterone acetate has better efficacy in restoring menstrual cycles and decreasing circulating testosterone concentrations. On the contrary, insulin sensitizers are superior to oral contraceptives in decreasing insulin indices and other insulin related complains of PCOS women.

Future Direction

Although the actual mechanism of PCOS development has not been clear enough, further investigations on the anti-cancer activity of metformin drug should be undertaken. Clinical trials including the effects of combined drugs like ethinyl estradiol and desogestrel or any other anti-diabetic drugs are mostly beneficial for clinicians as well as researchers in this certain area. We believe that this research will be beneficial for both scientists and students.

References

- Baillargeon, J. P., Jakubowicz, D. J., Iuorno, M. J., Jakubowicz, S., & Nestler, J. E. (2004). Effects of metformin and rosiglitazone, alone and in combination, in nonobese women with polycystic ovary syndrome and normal indices of insulin sensitivity. *Fertility and Sterility*, *82*(4), 893–902.
- Bhattacharya, S. M., Ghosh, M., & Basu, R. (2012). Effects of ethinyl estradiol and desogestrel on clinical and metabolic parameters in Indian patients with polycystic ovary syndrome. *Journal of Obstetrics and Gynaecology Research*, *38*(1), 285–290.
- Cetinkalp, S., Karadeniz, M., Erdogan, M., Ozgen, G., Saygl, F., & Ylmaz, C. (2009). The effects of rosiglitazone, metformin, and estradiol-cyproterone acetate on lean patients with polycystic ovary syndrome. *Endocrinologist*, *19*(3), 94–97.
- De Leo, V., Fruzzetti, F., Musacchio, M. C., Scolaro, V., Di Sabatino, A., & Morgante, G. (2013). Effect of a new oral contraceptive with estradiol valerate/dienogest on carbohydrate metabolism. *Contraception*, *88*(3), 364–368.
- De Leo, V., Musacchio, M. C., Morgante, G., Piomboni, P., & Petraglia, F. (2006). Metformin treatment is effective in obese teenage girls with PCOS. *Human Reproduction*, *21*(9), 2252–2256.
- Dereli, D., Dereli, T., Bayraktar, F., Ozgen, A. G., & Yilmaz, C. (2005). Endocrine and metabolic effects of rosiglitazone in non-obese women with polycystic ovary disease. *Endocrine Journal*, *52*(3), 299–308.
- Escobar-Morreale, H. F. (2018). Polycystic ovary syndrome: Definition, aetiology, diagnosis and treatment. *Nature Reviews Endocrinology*, *14*(5), 270–284.
- Fruzzetti, F., Perini, D., Lazzarini, V., Parrini, D., Gambacciani, M., & Genazzani, A. R.

- (2010). Comparison of effects of 3 mg drospirenone plus 20 µg ethinyl estradiol alone or combined with metformin or cyproterone acetate on classic metabolic cardiovascular risk factors in nonobese women with polycystic ovary syndrome. *Fertility and Sterility*, 94(5), 1793–1798.
- Goodarzi, M. O., & Azziz, R. (2006). Diagnosis, epidemiology, and genetics of the polycystic ovary syndrome. *Best Practice and Research: Clinical Endocrinology and Metabolism*, 20(2), 193–205.
- Kar, S. (2012). Clomiphene citrate or letrozole as first-line ovulation induction drug in infertile PCOS women: A prospective randomized trial. *Journal of Human Reproductive Sciences*, 5(3), 262–265.
- Karabulut, A., Demirlenk, S., & Şevket, O. (2012). Effects of ethinyl estradiol-cyproterone acetate treatment on metabolic syndrome, fat distribution and carotid intima media thickness in polycystic ovary syndrome. *Gynecological Endocrinology*, 28(4), 245–248.
- Kriplani, A., Periyasamy, A. J., Agarwal, N., Kulshrestha, V., Kumar, A., & Ammini, A. C. (2010). Effect of oral contraceptive containing ethinyl estradiol combined with drospirenone vs. desogestrel on clinical and biochemical parameters in patients with polycystic ovary syndrome. *Contraception*, 82(2), 139–146.
- Legro, R. S., Zaino, R. J., Demers, L. M., Kunesman, A. R., Gnatuk, C. L., Williams, N. I., & Dodson, W. C. (2007). The effects of metformin and rosiglitazone, alone and in combination, on the ovary and endometrium in polycystic ovary syndrome. *American Journal of Obstetrics and Gynecology*, 196(4), 402.e1-402.e11.
- Lemay, A., Dodin, S., Turcot, L., Déchêne, F., & Forest, J. C. (2006). Rosiglitazone and ethinyl estradiol/cyproterone acetate as single and combined treatment of overweight women with polycystic ovary syndrome and insulin resistance. *Human Reproduction*, 21(1), 121–128.

- Li, X., Guo, Y. R., Lin, J. F., Feng, Y., Billig, H., & Shao, R. (2014). Combination of Diane-35 and metformin to treat early endometrial carcinoma in PCOS women with insulin resistance. *Journal of Cancer*, *5*(3), 173–181.
- Luque-Ramírez, M., Álvarez-Blasco, F., Botella-Carretero, J. I., Martínez-Bermejo, E., Lasunción, M. A., & Escobar-Morreale, H. F. (2007). Comparison of ethinyl-estradiol plus cyproterone acetate versus metformin effects on classic metabolic cardiovascular risk factors in women with the polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*, *92*(7), 2453–2461.
- Luque-Ramírez, M., Mendieta-Azcona, C., Álvarez-Blasco, F., & Escobar-Morreale, H. F. (2009). Effects of metformin versus ethinyl-estradiol plus cyproterone acetate on ambulatory blood pressure monitoring and carotid intima media thickness in women with the polycystic ovary syndrome. *Fertility and Sterility*, *91*(6), 2527–2536.
- Mitkov, M., Pehlivanov, B., & Terzieva, D. (2005). Combined use of metformin and ethinyl estradiol-cyproterone acetate in polycystic ovary syndrome. *European Journal of Obstetrics and Gynecology and Reproductive Biology*, *118*(2), 209–213.
- Morin-Papunen, L. C., Vauhkonen, I., Koivunen, R. M., Ruokonen, A., Martikainen, H. K., & Tapanainen, J. S. (2000). Endocrine and metabolic effects of metformin versus ethinyl estradiol-cyproterone acetate in obese women with polycystic ovary syndrome: A randomized study. *Journal of Clinical Endocrinology and Metabolism*, *85*(9), 3161–3168.
- Naka, K. K., Kalantaridou, S. N., Bechlioulis, A., Kravariti, M., Kazakos, N., Katsouras, C. S., ... Michalis, L. K. (2011). Effect of ethinylestradiol/cyproterone acetate on endothelial function in young non-obese women with polycystic ovary syndrome: A pilot study. *Gynecological Endocrinology*, *27*(9), 615–621.
- Nawrocka, J., & Starczewski, A. (2007). Effects of metformin treatment in women with

- polycystic ovary syndrome depends on insulin resistance. *Gynecological Endocrinology*, 23(4), 231–237.
- Ortega-González, C., Luna, S., Hernández, L., Crespo, G., Aguayo, P., Arteaga-Troncoso, G., & Parra, A. (2005). Responses of serum androgen and insulin resistance to metformin and pioglitazone in obese, insulin-resistant women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*, 90(3), 1360–1365.
- Özdemir, S., Görkemli, H., Gezginç, K., Özdemir, M., & Kiyici, A. (2008). Clinical and metabolic effects of medroxyprogesterone acetate and ethinyl estradiol plus drospirenone in women with polycystic ovary syndrome. *International Journal of Gynecology and Obstetrics*, 103(1), 44–49.
- Palomba, S., Orio, F., Falbo, A., Manguso, F., Russo, T., Cascella, T., ... Zullo, F. (2005). Prospective parallel randomized, double-blind, double-dummy controlled clinical trial comparing clomiphene citrate and metformin as the first-line treatment for ovulation induction in nonobese anovulatory women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*, 90(7), 4068–4074.
- Rautio, K., Tapanainen, J. S., Ruukonen, A., & Morin-Papunen, L. C. (2005). Effects of metformin and ethinyl estradiol-cyproterone acetate on lipid levels in obese and non-obese women with polycystic ovary syndrome. *European Journal of Endocrinology*, 152(2), 269–275.
- Rautio, K., Tapanainen, J. S., Ruukonen, A., & Morin-Papunen, L. C. (2006). Endocrine and metabolic effects of rosiglitazone in overweight women with PCOS: A randomized placebo-controlled study. *Human Reproduction*, 21(6), 1400–1407.

